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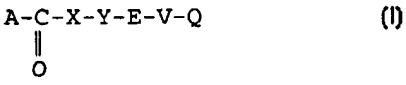

(I)

(57) Abstract

The present invention relates to compounds of formula (I) or formula (II) or pharmaceutically acceptable salts thereof useful for the prophylaxis and treatment of degenerative bone disorders, wherein A is (a), B is (b) and X, Y, E, V, Q, $R_1$, $R_2$, $R_3$, $R_4$, $R_5$ and $R_6$ are as defined in the specification.

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# BONE TARGETING AGENTS FOR OSTEOPOROSIS

This present application relates to novel compounds useful for the treatment and prophylaxis of degenerative bone disorders, such as osteoporosis.

Bone is a dynamic tissue, consisting of cells in a protein matrix, upon which is superimposed a crystalline structure of various calcium salts. Obviously, the bone skeleton serves as the rigid support for the body. In addition, bone is an organ which responds to hormones, and bone cells are able to solubilize the calcium salts in bone for use elsewhere in the body. This is a normal regulatory function of bone.

Diseases of excessive bone degeneration exist, including Paget's disease of bone and osteoporosis. The mechanisms are not well understood. In addition, the treatments available are, in general, combinations of endocrine, dietary and pharmacological treatments which are often unsuccessful. Clinical osteoporosis is found in approximately 25% of post-menopausal women, and subclinical osteoporosis, which is responsible for untold numbers of bone fractures in elderly women, is far more widespread.

Treatment of these disease states requires an agent that exerts an anabolic effect on bone or an anticatabolic effect thereon or both.

The mechanisms by which bone cells remodel bone have been extensively studied but are not clearly defined. One likely scenario is that resorption is caused by the secretion by bone cells of acid and proteolytic enzymes. For these enzymes to have their effect, it is likely that the tissue must be decalcified first. Following resorption, anabolic processes create new bone in the region. The initiating step is thought to be the acidification of the internal environment of bone, which is responsible for decalcification. One such acid, which has been implicated for many years in these processes, is carbonic acid.

Assuming carbonic acid, which is generated by the enzyme carbonic anhydrase, is involved in bone resorption, then administration of a drug which inhibits carbonic anhydrase should inhibit the liberation of calcium from bone in response to PTH.

This is indeed the case, as was first demonstrated in mammals by Waite, et al. in the publication entitled, "Inhibition of Bone Resorption by Acetazolamide in the Rat", Endocrinology, 87:1129(1970). One of the models used was the Induced Secondary Hyperparathyroid Rat (ISHR). ISHRs were prepared by surgical ligation of the renal arteries. In the rat, the kidney is responsible for the metabolism of citrate.

Upon ligation of the renal arteries, blood citrate concentration increases. Citrate chelates calcium and, while total calcium concentration is not affected by this binding, the amount of ionized calcium declines. This drop in plasma ionized calcium is the signal for the release of PTH. PTH, once released, signals bone to begin the resorptive process.

As one would predict, in the ISHR, the increased release of PTH leads to an increase in total plasma calcium concentration. Administration of the carbonic anhydrase inhibitor acetazolamide to ISHR completely inhibits this response.

Using classic endocrine ablation/replacement studies, it has been shown that this effect is indeed due to a response to PTH. If the ISHR rat has the parathyroid glands removed, the expected increase in plasma calcium concentration is not observed. In this same animal (ISHR without parathyroids), however, administration of PTH evokes the response, while acetazolamide and other heterocyclic sulfonamides (carbonic anhydrase inhibitors) abolish it.

Later work in tissue culture showed that inhibition of PTH-induced resorption by acetazolamide is due to a direct interaction at the level of bone ("Carbonic Anhydrous and Bone Remodeling: Sulfonamide Inhibition of Bone Resorption in Organ Culture", Minkin and Jennings, Science, (June 1970)).

These studies would lead one to suggest that acetazolamide would be useful as an inhibitor of bone resorption.

However, when one administers acetazolamide or other heterocyclic sulfonamide carbonic anhydrase inhibitors to normal animals, no change in plasma calcium concentration is observed. It has been demonstrated that the reason for this is that acetazolamide, while inhibiting calcium dissolution from bone due to the PTH response, also causes a systemic acidosis which concomitantly increases the breakdown of bone. These two competing effects mask one another. (See, "Acidosis Inhibits the Hypocalcemic Effect of Acetazolamide", Lineberry and Waite, Pharmacol. Exp. Ther., 211:452 (1979)).

Since these original studies, several other factors relative to bone resorption have been determined. For example, the following have subsequently been observed: heterocyclic sulfonamides such as acetazolamide which inhibit carbonic anhydrase also inhibit bone resorption; these sulfonamides have both effects (carbonic anhydrase inhibition and inhibition of bone resorption) at the same concentrations; heterocyclic sulfonamides which do not inhibit carbonic anhydrase do not inhibit bone resorption; heterocyclic sulfonamides which inhibit carbonic anhydrase also inhibit the bone resorptive effects of large doses of Vitamin D; and since other

parameters of bone metabolism are not affected by the sulfonamides, this is not a simple toxicity to bone cells.

There have been recent advances in this art. For example, in U.S. Patent No. 5,641,762 to Pierce, et al. it is disclosed that osteostats consisting of a bone seeking agent and a carbonic anhydrase inhibitor would be useful in the prophylaxis and treatment of degenerative bone disorders. The teaching therein exemplified compounds containing a carbonic anhydrase inhibitor moiety, e.g., sulfonamides and imidazoles and a bone seeking moiety, e.g., tetracycline and diphosphonates, which optionally are separated by a bridging agent. It also disclosed Mithramycin as a useful reagent which exhibits inhibition of bone resorption. It provided examples which were designated tetracycline-internally-active acetazolamide (TIA), tetracycline-internally active ethoxzolamide (TIE), tetracycline active acetazolamide, tetracycline active ethoxzolamide Δ-1, tetracycline active ethoxzolamide Δ-2, and aminohexyldiphosphonate-active-acetazolamide. The present inventors have now found other compounds useful for the prophylaxis and treatment of degenerative bone disorders.

These compounds are not based on tetracycline or diphosphonate; yet they are still bone-targeted. The compounds of the present invention are used in diagnosis

of bone disease or in treatment- exerting anabolic and/or anti-catabolic effects on bone.

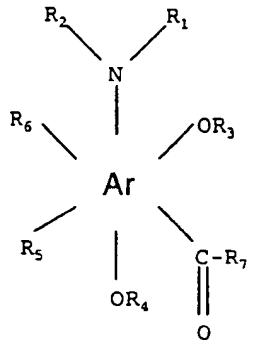
It is therefore one object of the present invention to provide novel compounds and their pharmaceutically acceptable compositions which are useful in the diagnosis, prophylaxis and treatment of degenerative bone disorders.

Another object of this invention is to provide a method for the preparation for these novel compounds.

A further object of the present invention is to provide a method for the diagnosis, treatment and prophylaxis of degenerative bone disorders, including osteoporosis.

These and other objects are achieved by providing novel compounds which are particularly characterized by active moieties as a part of first and second molecular domains linked together through a bridging group.

The first of these molecular domains is a bone targeting agent, i.e., an agent which has an affinity for the extracellular inorganic matrix of bone. The present invention contemplates a specific example of a bone targeting agent which binds to the mineral phase of bone. It is a compound of the formula:




III

wherein

$R_1$ and $R_2$ are independently hydrogen, lower alkyl or aryl lower alkyl,

$R_3$ is hydrogen or lower alkyl,

$R_4$ is hydrogen, lower alkyl, aryl lower alkyl or aryl,

$R_5$ and $R_6$ are on adjacent carbon atoms of Ar and are independently hydrogen or lower alkyl, or $R_5$ and $R_6$ taken together with the carbon atoms to which they are attached form a ring containing up to 10 ring carbon atoms and up to a total of 18 carbon atoms,

$R_7$ is hydroxy, lower alkoxy or $NR_8R_9$ and $R_8$ and $R_9$ are independently hydrogen or lower alkyl, and

Ar is aryl comprised of carbon and hydrogen atoms having 6-10 ring carbon atoms or is the corresponding saturated or partially unsaturated cyclic moiety.

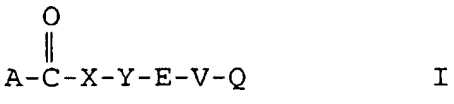
The second of these molecular domains is a specific bone active domain, which effects bone metabolism by either increasing bone formation or inhibiting bone resorption or both. The bone active domains contemplated by the present invention include compounds which decrease bone resorptions such as specific steroids, especially sex hormones, e.g., androgens and estrogens. It also includes DHEA (3β-hydroxy-5-androsten-17-one) and derivative thereof and prostaglandins. Other examples contemplated by the present invention include proton pump inhibitors and carbonic anhydrase inhibitors. Other examples contemplated by the present invention include compounds which increase bone formation, such as hormones especially parathyroid hormones and the thyroid hormones, especially O-(4-hydroxy-3,5-diiodophenyl)-3,5-diiodo-2-tyrosine (also known as thyroxine or ($T_4$)) and O-(4-hydroxy-3-iodophenyl-3,5-diiodo-L-tyrosine (also known as liothyronine or ($T_3$)), and pharmaceutically acceptable salts thereof, such as Group IA salts of $T_3$ or $T_4$ (e.g., sodium, potassium) and the like. Other bone active domains contemplated by the present invention include compounds which tend to increase bone formation and decrease bone resorption. Examples of these include androgens, HMG CoA reductase inhibitors, and the like.

If the bone active domain is a sex hormone, it is preferred that the sex hormones are steroids.

Preferably, the sex hormones are estrogens, estrogen precursors or androgens.

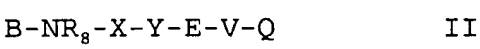
The present invention contemplates that the second domain is linked to the first molecular domain through a bridging group. More specifically, the two domains are separated by a bridging group which has functional moieties at both of its respective ends, that is, groups which react with the bone targeting agents and with the bone active agents.

The present invention is directed to compounds of the formula:



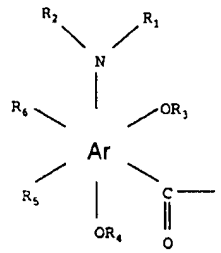
A-C-X-Y-E-V-Q I

or



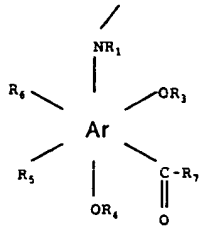
B-NR$_8$-X-Y-E-V-Q II

or pharmaceutically acceptable salts thereof wherein B is




;

A is




;

Ar is aryl comprised of carbon and hydrogen atoms having 6-10 ring carbon atoms or the corresponding saturated or partially unsaturated cyclic group;

$R_1$ is hydrogen, lower alkyl or aryl lower alkyl;

$R_2$ is hydrogen, lower alkyl or aryl lower alkyl;

$R_3$ is hydrogen or lower alkyl;

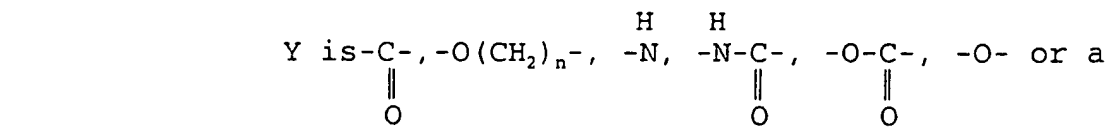
$R_4$ is hydrogen, aryl lower alkyl, aryl or lower alkyl;

$R_5$ and $R_6$ are substituted on adjacent carbon atoms of Ar, and are independently hydrogen or lower alkyl or $R_5$ and $R_6$ taken together with the carbon atoms to which they are attached form a ring containing 4-10 ring carbon atoms and up to a total of 18 carbon atoms;

$R_7$ is hydroxy, lower alkoxy, or $NR_8R_9$;

$R_8$ and $R_9$ are independently hydrogen or lower alkyl;

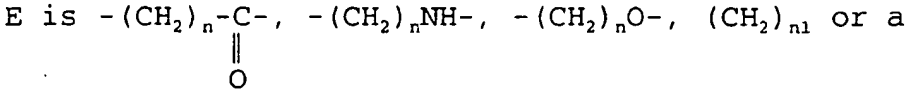
X is an alkylene group containing from 1-10 carbon atoms on the main chain and up to a total of 20 carbon atoms, or X is a chemical bond;

Y is 

or a chemical bond;

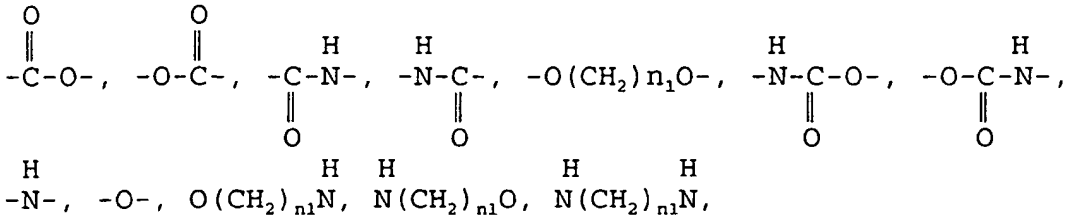
V is 

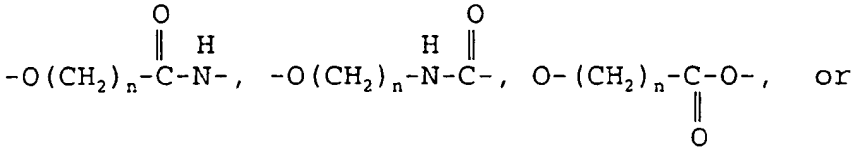
or -O-;

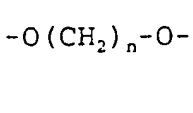
E is 

or a chemical bond;

provided Y-E-V is




QVH is a bone active domain;

Q is the bone active domain less a VH functional group thereon or a functional group capable of being converted to VH, said bone active domain being selected from the group consisting of a carbonic

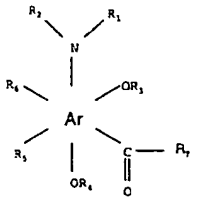
anhydrase inhibitor, a sex hormone such as estrogens and androgens, which optionally may be substituted with a phosphate group, Vitamin D, or other steroids which exhibit androgen or estrogen effects, such as DHEA, and the like, a proton pump inhibitor, HmG CoA reductase inhibitor, parathyroid hormone, $T_3$, $T_4$, prostaglandin and mixtures thereof and pharmaceutically acceptable salts thereof, wherein V is bonded to the carbon atom devoid of said functional group;

n is 0-6;and

$n_1$ is 1-6.

The present invention is also directed to the pharmaceutical compositions containing a pharmaceutically effective amount of the compounds of Formula I or II. In addition, the present invention is directed to a method for the treatment of degenerative bone disorders in an animal, such as mammals, including cats, dogs, horses, rabbits, rats and especially humans comprising administering to the animal in need of such treatment a pharmaceutically effective amount of the compound of Formula I or II.

The present invention is also directed to compounds of Formula III having the formula:




III

wherein $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$, $R_7$ and Ar are as defined hereinabove. Compounds of Formula III are intermediates in the preparation of compounds of Formula I and II.

Figure 1 is a graphical representation depicting the effect of various dosages of BTE2-D1, BTE2-D2, BTE2-D3 on the density of the distal femur in female rat as compared to two controls, estradiol and raloxifene.

Figure 2 is a graphical representation depicting the effect of various dosage of BTE2-D2 on the distal femoral strength of female rats as compared to raloxifene and control.

As described hereinabove, the present invention is directed, inter alia, to compounds for the treatment and prophylaxis of degenerative bone disorders. These novel compounds are defined by the compounds of Formula I or II.

As used herein, the term "lower alkyl", when used alone or in combination, refers to alkyl groups containing 1-6 carbon atoms. They may be straight-chained or branched. Examples include methyl, ethyl, propyl, isopropyl, n-butyl, sec-butyl, t-butyl, isobutyl, n-pentyl, isopentyl, neopentyl, n-hexyl, and the like. It is preferred that the alkyl group contains 1-3 carbon atoms. The most preferred alkyl group is methyl.

The term "alkylene", as used herein, is a bridging group that contains 1-20 carbon atoms which, for purposes of this application, is preferably a straight chain. However, the alkylene group may be branched, i.e., they are alkyl substituted. As used herein, the total number of carbon atoms on the alkylene chain, including the main chain in the bridging group, ranges from 1-20 carbon atoms. In addition, the alkylene group may be substituted with other groups such as hydroxy, amino, loweralkylamino or diloweralkylamino. It is preferred that the alkylene chain contains 1-6 carbon atoms and it is more preferred that it contains 2-4 carbon atoms. It is even more preferred that the alkylene be straight chained.

"Aryl", when used alone or in combination with other groups, refers to an aromatic group containing only ring carbon atoms and having 6-14 ring carbon atoms and up to a total of 18 carbon atoms. Examples include phenyl, α-naphthyl, β-naphthyl, tolyl, xylyl, and the like.

The term aryl or the corresponding saturated or partially unsaturated cyclic group of aryl refers to an aryl group as defined hereinabove or the corresponding cyclic group containing the same number of carbon atoms and identical substituents thereon, but containing less carbon-carbon double bonds thereon. For example, if the aryl group is phenyl, the corresponding saturated or partially saturated group is 1,3-

cyclohexenyl, 1-cyclohexenyl, or cyclohexyl. Similarly, if the aryl group is tolyl, the corresponding partially unsaturated or saturated cyclic group is methylcyclohexyl, 1-methyl-1-cyclohexenyl, 4-methyl-1-cyclohexenyl, 3-methyl-1-cyclohexenyl, 1-methyl-2,4-cyclohexadienyl, 1-methyl-1-,3-cyclohexadienyl or 1-methyl-1,5-cyclohexadienyl. All of these cyclic structures are contemplated to be included in the compounds of the present invention. Moreover, if aryl is naphthyl, the corresponding saturated or partially unsaturated cyclic group includes decalin and the corresponding two ring fused cyclic compound containing $C_{10}$ ring carbon atoms containing 1, 2, 3, or 4 double bonds as well as naphthyl. These are all contemplated to be within the scope of Ar.

Halo refers to the halogen group in the periodic table. Examples include F, Cl, Br and I.

Lower alkoxy refers to an alkyl group, as defined herein, connected to the main chain through an oxygen bridging atom. Examples include methoxy, ethoxy, propoxy, i-propoxy, butoxy, i-butoxy, sec-butoxy, t-butoxy and the like.

Similarly, "aryloxy", as defined herein refers to an aryl group connected to the main chain by an oxygen bridging atom. Examples include phenoxy,

naphthoxy, and methyl phenoxy, (e.g., 4-methyl phenoxy), and the like.

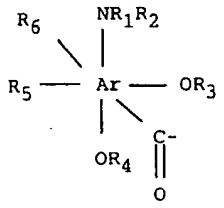
Moreover, lower "arylalkoxy" refers to a lower arylalkyl group, as defined herein, linked to the main chain by an oxygen bridging group. Examples include benzyloxy, phenethoxy, and the like.

"Lower arylalkyl" refers to an aryl group bonded to a bridging alkyl group, as defined herein. Examples include benzyl, phenethyl, naphthylethyl, and the like.

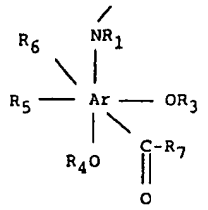
As described hereinabove, an aspect of the present invention is directed to a compound of the formula:

or pharmaceutically acceptable salts thereof, wherein X, Y, E, V, $R_8$ and Q are as defined hereinabove.

B is a moiety of the formula:

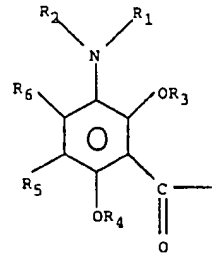


and A is a moiety of the formula:



wherein $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$ and $R_7$ are as defined hereinabove and Ar is aryl comprised of carbon and hydrogen atoms having 6-10 carbon ring atoms or the corresponding saturated or partially unsaturated moiety. It is to be noted that $R_5$ and $R_6$ are substituents on adjacent carbon atoms of the Ar ring.

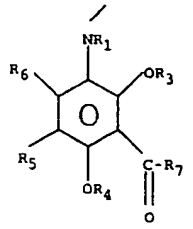
The A and B moieties have the substituents indicated hereinabove. However, the Ar group may vary, i.e., it may be fully saturated or partially saturated, for example, it may be cyclohexyl or cyclohexenyl or cyclohexadienyl. It is preferred that Ar is phenyl, napthyl or cyclohexyl, cyclohexenyl or cyclohexadienyl with cyclohexyl, cyclohexenyl or cyclohexadienyl and phenyl being even more preferred. Thus, the most preferred B is a moiety having the formula:



, or the corresponding cyclohexyl, cyclohexenyl or the cyclohexadienyl

VI

while the more preferred A has the formula



VII

or the corresponding cyclohexyl, cyclohexenyl, or the cyclohexadienyl

wherein

$R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$ and $R_7$ are as defined hereinabove.

The most preferred values of Ar for both A and B is cyclohexyl and especially phenyl.

In the formulae of Compounds I and II, Y, E and V represent the functionalities that bridge Q with X, as defined hereinabove. The type of functionalities of Y, E, V, and YEV are defined hereinabove, but it is preferred that

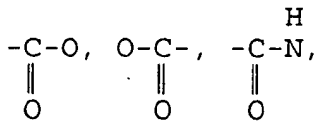
YEV is -C(=O)-O-, -O-C(=O)-, -C(=O)-N(H)-, -N(H)-C(=O)-, -N(H)-

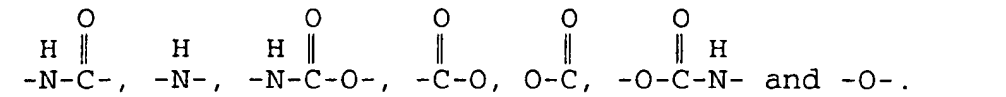
H H H

-N(H)-C(=O)-O-, -O-C(=O)-N(H)-, -O-$(CH_2)_n$-C(=O)-N(H)-, -O-,

H

-O$(CH_2)_n$N(H)-C(=O)-, -O-$(CH_2)_n$-C(=O)-O-, or -O-$(CH_2)_n$-O-C(=O)-.

The most preferred YEV is  , , ,



It is preferred that n is 1-6 and more preferably n is 1-4.

It is preferred that $n_1$ is 1-4 and especially 1 or 2.

The bone active domain is a compound which increases bone formation or decreases bone resorption or both and is defined as the groups described herein. Examples, as defined herein, includes sex hormones (estrogens and androgens), DHEA and derivatives thereof carbonic anhydrase inhibitors, Vitamin D derivatives, $T_3$, $T_4$, proton pump inhibitors, parathyroid hormones, HMG CoA reductase inhibitors and prostaglandins, and pharmaceutically acceptable salts thereof.

Examples of estrogens useful in the present invention are estradiol, estrone, estriol, and the like.

Examples of androgens useful in the present invention include testosterone, 5 α-dihydro- testosterone, androstenedione, etiocholanolone, epiandrosterone, androsterone, 17 α-methyl testosterone, fluoxymesterone, 17 α-ethyltestosterone, 17 α-methylandrostan- 3β, 17β-diol, androstan-3α, 17β-diol, androstan- 3α-17α-diol, androstan- 17β-ol-3-one, androstane- 17α-ol-3-one, $\Delta^5$-androsten-3α, 17β-diol, $\Delta^5$-androstene-3β, 17β-diol, Androstane-3-17-dione, $\Delta^4$- androstenedione, and the like.

Examples of the family of D vitamins useful in the present invention include ergocalciferol, (vitamin D2), cholecalciferol (vitamin $D_3$), 25-hydroxy-ergocalciferol, 1,25-dihydroxyergo- calciferol, 25-hydroxy-cholecalciferol, 1,25-dihydroxycholecalciferol, 24, 25 dihydroxy vit D3 and the like.

Examples of various carbonic anhydrase inhibitors have been set forth in U.S. Patent No. 5,641,762, 5,242,937, 5,055,480, and 5,059,613, all of which are to Pierce, the contents of all of which are incorporated by reference.

Specific examples of carbonic anhydrase inhibitors include acetazolamide, 2-amino-1,3,4-thiadiazole-5-sulfonamide, 6-hydroxy-2-benzothiazole sulfonamide, 6-ethylsuccinyloxy-2-benzothiazole sulfonamide, succinylazolamide, oxaloylazolamide, and the like.

HMG-CoA reductase inhibitors are those compounds which inhibit HMG-CoA reductase and exert an anabolic effect on bone. Examples of the HMG-CoA reductase inhibitors are lovastatin, compactin, simvastatin, prevastatin, mevastatin and the like and pharmaceutically acceptable salts thereof.

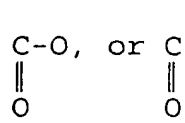
Examples of proton pump inhibitors contemplated by the present invention include O-desmethylomeprazole and the like.

Examples of the thyroid hormones include $T_3$, $T_4$ and pharmaceutically acceptable salts thereof.

Examples of prostaglandins include prostaglandin E-2, prostaglandin E-1, prostaglandin $F_{2\alpha}$, 15-methyl-$PGE_2$, 15-methyl-11-deoxy-$PGE_1$, and the like, other cyclooxygenase products derived from eicosatetraeneoic (22:4) acid and the corresponding 22:5 and 22:6 analogs and the like.

The bone active domain, as defined herein, contains an hydroxy group, an amino group, a carboxy group or a group that can be converted to a hydroxy group, amino group, or carboxy group by chemical means. For example, if the bone active domain contains a carbonyl group, it can be reduced to the corresponding hydroxy group. As indicated herein, the hydroxy group can be oxidized to a carboxy group, and thus the linkage of Q to the bridging group may be through an

acyl group bonded to oxygen or nitrogen atom

(e.g.,  ; or the hydroxy group can

be reacted with an acid to form an ester, so that the linking of group Q to the bridging group may be  ; or the hydroxy group may be converted to an amine, making the linking group of Q to the bridging group NH or the amine may be reacted with an acid, making the

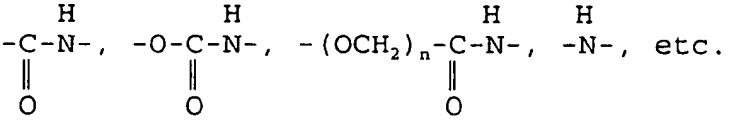
linking group of Q to the bridging group -N-C-. It is through the oxygen atom of the hydroxy group, or the acyl group bonded to an oxygen atom or nitrogen atom, etc. that the bone active domain is connected to the bridging group separating the bone active domains A or B, as defined herein.

As defined herein, the bone active domain contains the VH group, wherein V is C-O, -O- or NH. The bone active domain is bonded to the bridging group through V.

Q as defined herein, is the bone active domain without the VH group or the group that is converted to VH, through which Q is bonded to the bridging group XYE.

It is to be understood, that the oxygen atom from the hydroxy group or the carboxy group (C-O-), or nitrogen atom from the amino group is the V moiety. This group (V) is the moiety that forms the ether, amide or ester which permits the bridging group to bind to Q. The V group is thus attached to Q at the position wherein the carboxy group, amino group or hydroxy group or other group or a group that was converted to those groups was originally present. For example, if Q is less an amino

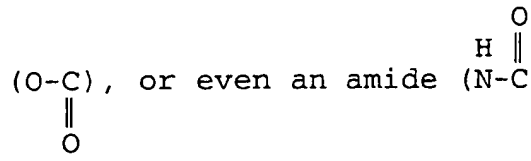
group then Q may be bonded to the bridging group as an amide and YEV is, among other things,



On the other hand, the bone active domain may be bonded to the bridging group through a reaction which converts the functional group thereon to VH; wherein VH was not originally present at the position of the bone active domain prior to the reaction. For example, the bone active domain may be bonded at the carbon to which is attached an oxo (  ) group. If the oxo group does not form a bond directly with the bridging group XYE, the oxo group originally present is chemically modified to a moiety which can react to form a bond with XYE. For example, the oxo group may be reduced by reagents known in the art, such as $H_2/Pd$, $NaBH_4$, $LiAlH_4$, amalgamated zinc and HCl, or hydrazine and a base such as KOH or potassium tert-butoxide to form the corresponding alcohol. The alcohol thus formed may be oxidized to the corresponding carboxylic acid by reagents known in the art, e.g., aqueous permanganate or chromic oxide, and the like and then reacted with a bridging group having an alcohol or amine moiety thereon under conditions known in the art

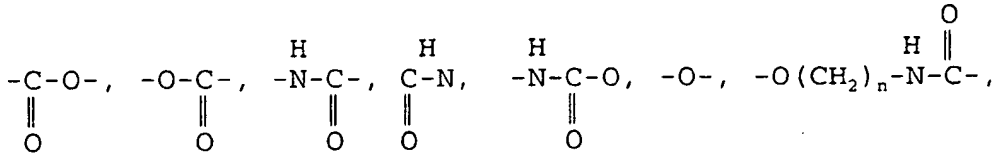
to form an ester or amide. Alternatively, the hydroxy group may react with a bridging group having an acylating moiety to form an ester or the hydroxy group may react with a bridging group under conditions known in the art to form an ether. Moreover, the hydroxy group may be converted to a leaving group, such as halide or sulfonic ester, such as brosylates, mesylates, or tosylates and the like, and the compound thus formed is then reacted with a bridging group containing an amine to form an amine linkage (NH). Or the compound in which the hydroxy group is converted to a leaving group may react with ammonia followed by strong base, such as hydroxide (e.g., sodium hydroxide) to form an amine. This amine may be the linking group to XYE or it, in turn, may react with a bridging group having a carboxy group thereon to form an amide (CONH) linkage. However, if YE contains an amine the oxo group may react directly with the amine under reductive amination conditions to form the imine which is then catalytically reduced by e.g., $H_2/N_1$ or sodium cyanohydridoborate, and the like.

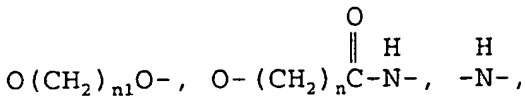
Similarly, if the bone active domain is less the hydroxy group, then it is bonded to the bridging group as an ester (C-O) or ether, or if the hydroxy group is oxidized to form the acid, as a different ester

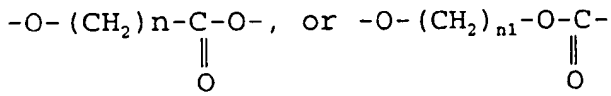


(O-C), or even an amide (N-C).

Thus, if the bone active domain is less an oxo or hydroxy group, then Q is bonded to the bridging group as an ester or ether or amide or amine and YEV is preferably







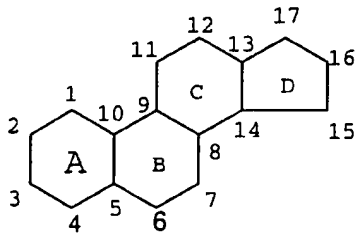
Thus, Q as defined herein is the bone active domain without the VH group or the group chemically transformed to VH, which group than reacts to form a bond with the bridging group.

The discussions hereinabove only refer to the moiety to which the bridging group is attached on the bone active domain. It is to be understood that the bone active domain may, in addition, contain an amino, hydroxy, carboxy, oxo or any other group that is capable of being converted to amino, hydroxy or carboxy.

A preferred bone active domain is a sex hormone, (e.g., estrogen or androgen). As used herein, an estrogen is a female sex hormone; it is a compound responsible for the development of the female secondary sex characteristics. An androgen, as used herein, is a male sex hormone.

Some sex hormones are steroids, e.g., estradiol, estrone, estriol and the like and the 3-phosphate derivatives thereof (all of which are estrogens) or testosterone, dihydroepiandrosterone (DHEA), androstenedione, etiocholanolone, epiandrosterone, androsterone, 17 α-methyl testosterone, fluoxymesterone, 17 α-ethyltestosterone, 17 α-methylandrostan-3β, 17β-diol, androstan-3α, 17β-diol, androstan- 3α-17α-diol, androstan- 17β-ol-3-one, androstane- 17α-ol-3-one, $\Delta^5$-androsten-3α, 17β-diol, $\Delta^5$-androstene-3β, 17β-diol, Androstane-3-17-dione, $\Delta^4$-androstenedione, and the like, and the 3-phosphate derivatives thereof (all of which are androgens). The most preferred androgens are testosterone, DHEA, androsterone, and 5α- dihydrotestosterone. Other sex hormones are synthetic and are non-steroidal, such as diethylstilbestrol or hexestrol. Both types of sex hormones are contemplated to be used in the present invention. The preferred sex hormones are steroidal estrogens and androgens.

Steroids, as used herein, are those compounds having a fused 17-carbon atoms ring system. They generally have the ring structure depicted hereinbelow:




That is, they have three-6 membered rings fused together, designated as the A, B and C ring and a 5-membered ring fused to the "C" ring (identified as the D ring). The above formula also indicates the conventional numbering in the steroidal ring. It is to be noted that the carbon backbone nucleus of estrogens have a methyl group substituted at C-13. The methyl group carbon is number C-18. On the other hand, the androgens have a methyl group substituted at C-10 and C-13. The methyl group carbons are numbered C-19 and C-18, respectively.

The steriods used in the present invention may contain double bonds in the A, B, C, and D rings or they may be completely saturated. Moreover, either the A or B ring or both may be aromatic. The preferred steroidal estrogens contain an aromatic A ring, and no other carbon-carbon double bonds. Moreover, the preferred

steroidal estrogens contain a methyl substituent at C-13 of the steroidal ring.

The preferred androgens have a methyl substituent at C-10 and C-13. Moreover, it is preferable that the C and D rings of either the androgens or estrogens contain no carbon-carbon double bond. The preferred androgens have either no carbon-carbon double bonds in the A or B ring or have one double bond in the A ring, preferably between C-4 and C-5 or a double bond in the B ring, preferably between C-5 and C-6. The 3-position of the steroidal ring of the estrogen used in the present invention is preferably hydroxy or phosphate, while the 3-position of the androgen used in the present invention is preferably hydroxy, phosphate or oxo. The steroid rings of the sex hormones may be unsubstituted or substituted wherein the substituents include such groups as lower alkyl, halo, hydroxy, lower alkoxy, amino, lower alkylamino, diloweralkylamino, and the like.

The D ring of the steroids of the sex hormones contain a C or C at C-17 of the steroid. In a preferred
‖ |
O OH
embodiment, the C-17 (hydroxy or oxo group) is bound through the oxygen atom to YE or the oxo or hydroxy group is converted to another group which is reactive with YE, so that the C-17 atom is bound to YE through said converted group, as defined herein. In a preferred

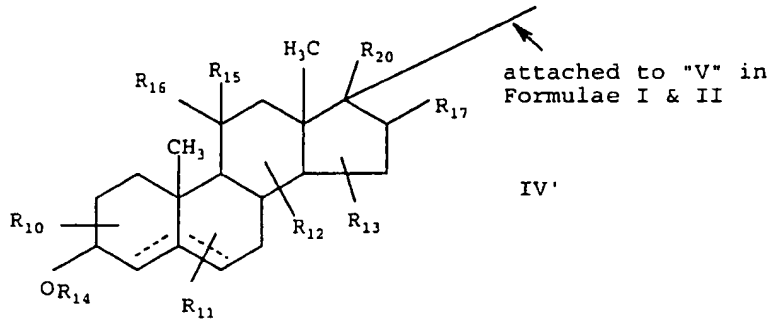
embodiment, Q will exclude hydroxy or oxo substituent at C-17. Thus Q, in the preferred embodiment defined herein includes DHEA, the androgens and estrogens without the substituents at C-17. Thus, in the preferred embodiment of the present intention, Y-E-V is bonded to C-17 of the steroid. If YEV forms a bond at C-17, the α-isomer may be formed, but it is preferred that the β-isomer is formed.

As used herein, the "V" moiety i.e., the moiety bonded directly to Q, corresponds to the "V" atom in the compounds of Formulae I and II described hereinabove. In a preferred embodiment, V is O. Thus, the preferred sex steroids used in the present invention have at least one hydroxy group (V-H, where V=O). Moreover, the preferred sex hormones have, in addition to the hydroxy group mentioned hereinabove, an $OR_{14}$ substituent, wherein $R_{14}$ is hydrogen, lower alkyl, aryl or lower arylalkyl or phosphate ($PO_3H_2$) or the pharmaceutically acceptable salts thereof. When Q is an androgen, $R_{14}$ is as defined hereinabove or additionally, $OR_{14}$ may be an oxo group, i.e., $(O)$. A preferred embodiment of Q groups has a steroid moiety backbone having at least one hydroxy group and an $OR_{14}$ substituent, wherein $OR_{14}$ is as defined hereinabove. In the steroid moiety, it is preferred that the hydroxy (V-H) group is a substituent on the D-ring, preferably at the 16-position and most preferably at the 17-position of the steroidal ring. Moreover, it is also preferred

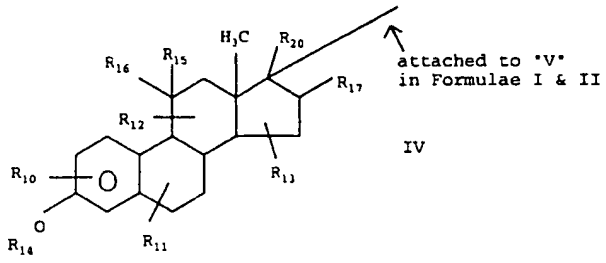
that the $OR_{14}$ is a substituent on the A ring and most preferably at the 3-position. Finally it is most preferred that Q is bonded to the V, e.g., O atom in the formula of compounds I and II at the 16- or 17-position, and most preferably at the 17-position of the steroidal ring.

The preferred values of $R_{14}$ are hydrogen and arylalkyl, especially benzyl, and phosphate. The most preferred values are hydrogen and phosphate.

In an embodiment of the present invention, it is preferred that Q has the formula:


attached to "V" in Formulae I & II
IV'

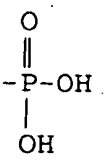
or


attached to "V" in Formulae I & II
IV

wherein

$R_{10}$, $R_{11}$, $R_{12}$ and $R_{13}$ are independently hydrogen, lower alkyl, lower alkoxy, hydroxy, halo, amino, loweralkylamino, and diloweralkylamino;

$R_{14}$ is hydrogen, lower alkyl, aryl or lower arylalkyl or



or when Q is an androgen, $OR_{14}$ may additionally be oxo;

$R_{15}$ and $R_{16}$ are independently hydrogen, lower alkyl, halo, hydroxy, lower alkoxy, amino, lower alkylamino, or diloweralkylamino, or $R_{15}$ and $R_{16}$ taken together form an =O; and

$R_{17}$ is hydrogen, lower alkyl, hydroxy, oxo lower alkynyl, lower alkenyl, halo, loweralkoxy, amino, lower alkylamino or diloweralkylamino;

$R_{20}$ is hydrogen, lower alkyl or lower alkynyl, and --- signifies that a carbon-carbon double bond may be present between C-4 and C-5 or C-5 and C-6 of the steroid ring, but not both. Additionally, the present invention contemplates compounds wherein neither the A ring or the B ring contains carbon-carbon double bonds.

In the moiety of Formula IV and IV, it is preferred that the carbon bond to VEY is through on β-linkage at the 17-position.

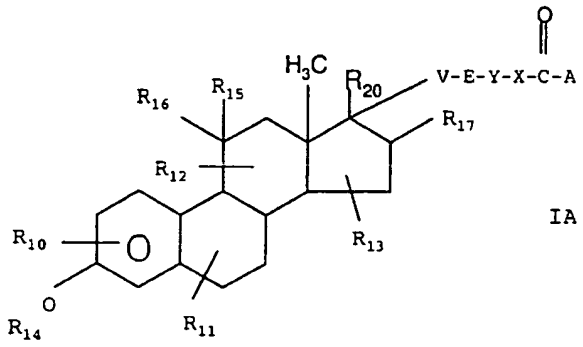
In the moiety of Formulae IV and IV', it is preferred, that $R_{10}$, $R_{12}$ and $R_{20}$ are each hydrogen. It is also preferred that $R_{13}$ is hydrogen or hydroxy. It is preferred that $R_{11}$ is hydrogen or hydroxy. If $R_{11}$ is hydroxy, it is preferred that $R_{11}$ is at the 6-position of the steroid.

It is preferred that $R_{15}$ and $R_{16}$ are hydrogen. It is also preferred that $R_{15}$ and $R_{16}$ taken together form an oxo group.

It is preferred that $R_{14}$ as depicted in Formula IV and IV' is hydrogen, or lower alkyl, e.g., methyl, or phosphate or a pharmaceutically acceptable salt thereof. If the steroid ring is an androgen, then $OR_{14}$ may additionally be oxo. The most preferred value of $R_{14}$ is hydrogen.

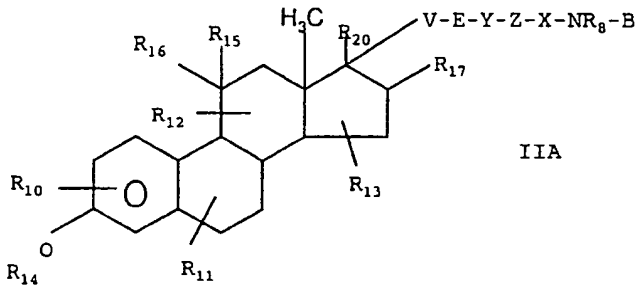
The preferred values of $R_{17}$ are hydroxy, lower alkoxy, lower alkynyl, e.g., ethynyl, and hydrogen. It is especially preferred that $R_{17}$ is lower alkoxy, e.g., methoxy, lower alkynyl, e.g., ethynyl, oxo and most especially hydroxy and hydrogen.

In the moiety of Formula IV and IV', it is to be understood that it is preferred that the bond at the 17-position is linked to the V atom of the compound of Formulae I and II. As an illustration, compounds of Formulae I and II may be written as



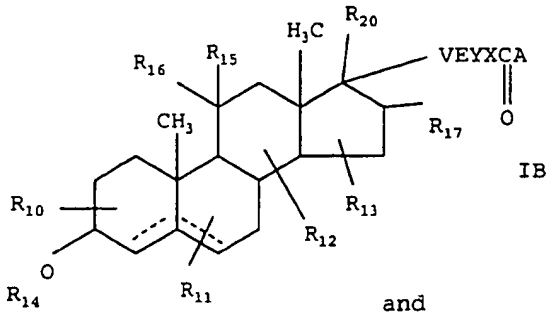

IA

and



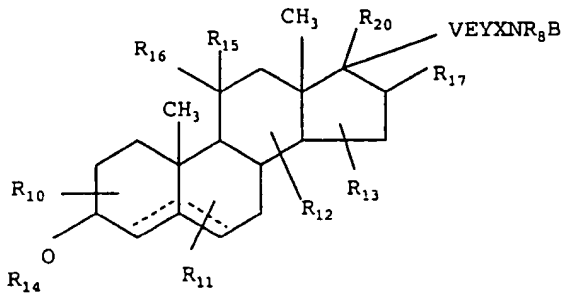

IIA

respectively. However, the corresponding androstane steroid derivatives are also contemplated to be encompassed within the scope of the present invention:



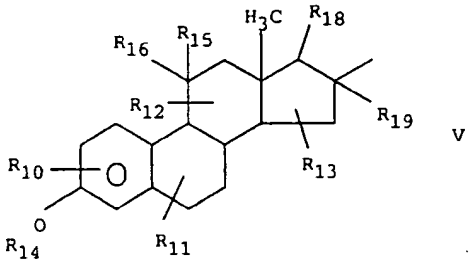

IB

and



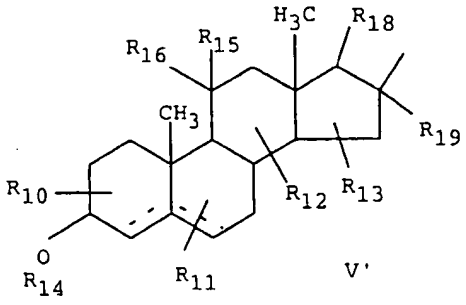

IIB

Another preferred embodiment of Q has the formula:




V

and




V'

wherein $R_{10}$, $R_{11}$, $R_{12}$, $R_{13}$, $R_{14}$, $R_{15}$, and $R_{16}$ and --- are as defined hereinabove, and $R_{18}$ is hydroxy, oxo, lower alkynyl, lower alkenyl, lower alkyl, hydrogen, lower alkoxy, halo, amino, lower alkylamino or diloweralkylamino, and $R_{19}$ is hydrogen, lower alkyl or lower alkynyl.

In the more preferred embodiment, it is preferred in compounds of Formulae IV, IV', V', or VI, IIA, II, IIA, IIB that the variable V is O.

In the embodiment of Q of Formula V or V' the linkage to V-E-Y-X-C-A of Formula I and V-E-Y-X-$NR_8$-B

of Formula II is at the 16-position of the steroid, and not the 17-position as in Formula IV or IV' hereinabove.

In the moiety of Formula V or V', the preferred values of $R_{10}$, $R_{11}$, $R_{12}$, $R_{13}$, $R_{14}$, $R_{15}$ and $R_{16}$ are as described hereinabove for the moiety of Formula IV or IV', respectively. Moreover, it is preferred that $R_{18}$ is hydroxy, lower alkoxy, lower alkynyl and hydrogen. Especially preferred values of $R_{18}$ is lower alkoxy, e.g., methoxy, lower alkynyl, e.g., ethynyl, oxo and most especially hydroxy or hydrogen.

The most preferred value of $R_{19}$ is hydrogen.

Although these are various embodiments in which the linkage to YE is at the 16 or 17 position of the steroid, other positions of the steroid ring be used to link to YE. For example, the linkage to YE may be at

the 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 position of the steroid. Of these positions, it is preferred that the linkage to YE is at the 6 position.

As depicted, A is bonded to  at the nitrogen atom which is bonded to $R_1$ while B is bonded to $NR_8$ at the acyl group  .

In the formula of A and B, it is preferred that $R_1$ is hydrogen or lower alkyl and it is especially preferred that $R_1$ is hydrogen.

$R_2$ is preferably hydrogen.

$R_3$ is preferably hydrogen or an alkyl group containing 1-3 carbon atoms or aryl lower alkyl, such as benzyl.

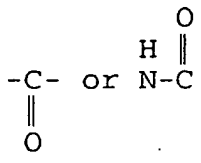
The preferred $R_4$, $R_5$, and $R_6$ are each hydrogen. However, in an embodiment of the present invention, $R_5$ and $R_6$ taken together may form a ring containing 6-14 ring carbon atoms. This ring may be monocyclic, bicyclic or tricyclic. In addition, the cyclic moiety may be saturated, partially unsaturated or aromatic.

It is preferred that $R_7$ is $NR_8R_9$.

The preferred $R_8$ and $R_9$ are each hydrogen.

As defined herein, X is an alkylene chain containing up to 10 carbon atoms in the main chain and up to a total of 20 carbon atoms. However, it is

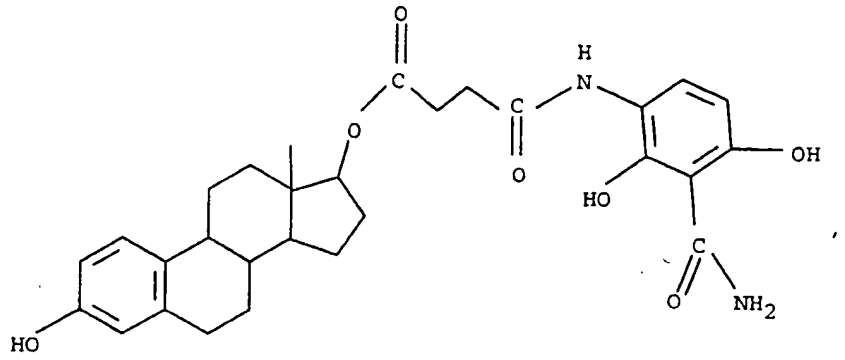
preferred that X contains a total of 1-6 carbon atoms and more preferably 1-3 carbon atoms. X may be straight chained or branched, but it is preferred that the alkylene chain is a straight chain. It is more preferred that X contains 2-4 carbon atoms, which although may be branched, but preferably is straight chained.

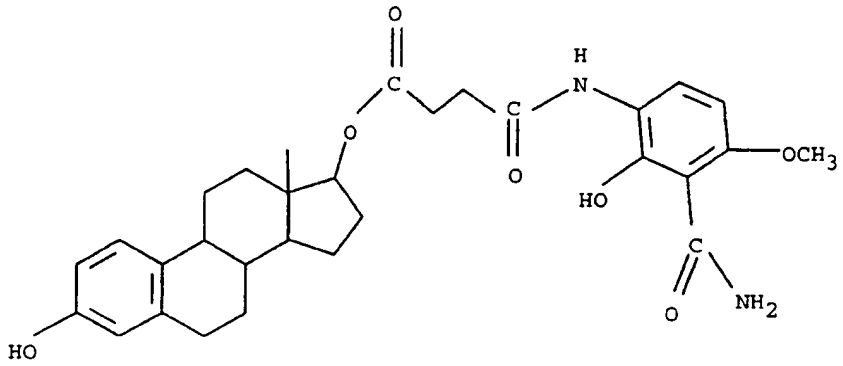
As defined herein, Y is preferably  or  or a chemical bond.

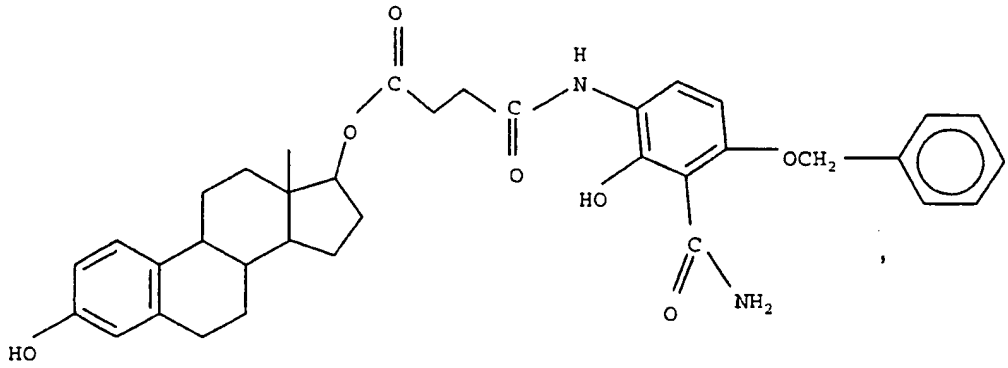
It is to be understood, for purposes of this application, that the acyl group is to be bonded to the oxygen atom (O) from the estrogen or androgen moiety. It is preferred that Y is  .

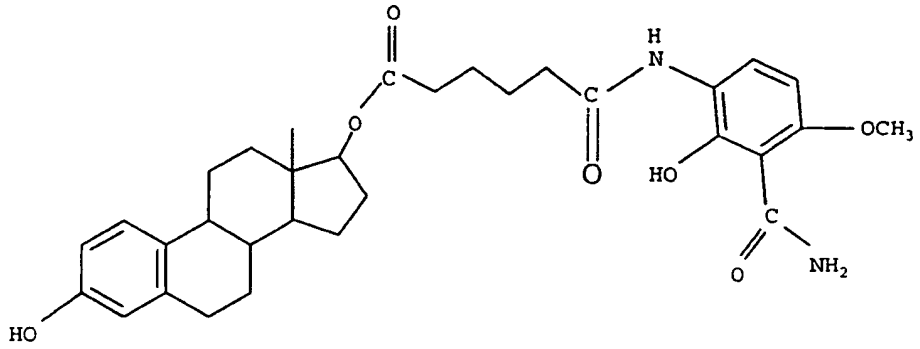
In the present invention, the compounds of Formula I are preferred.

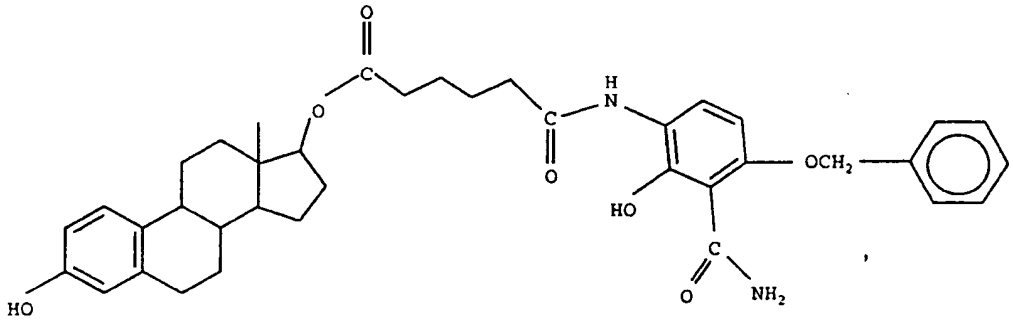
Preferred compounds of the present invention include the following:

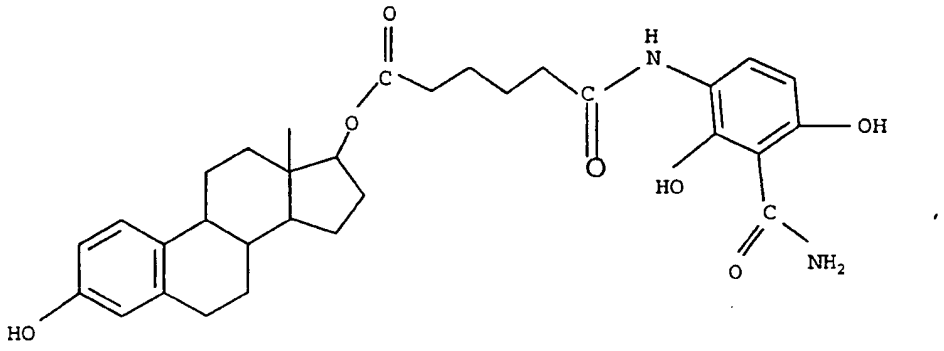


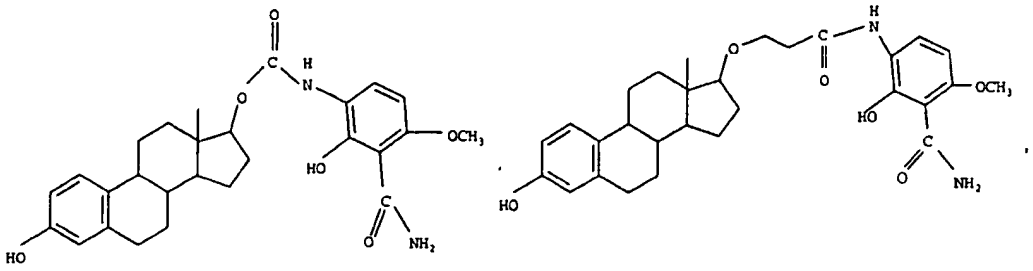


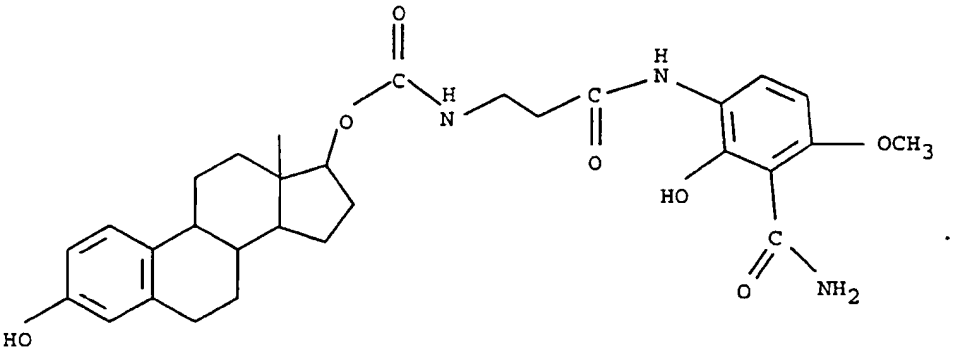


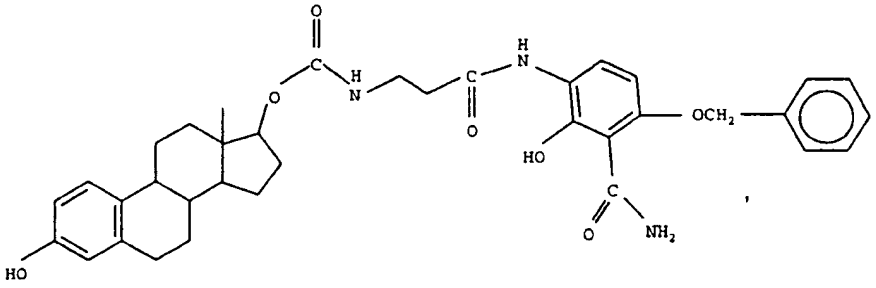


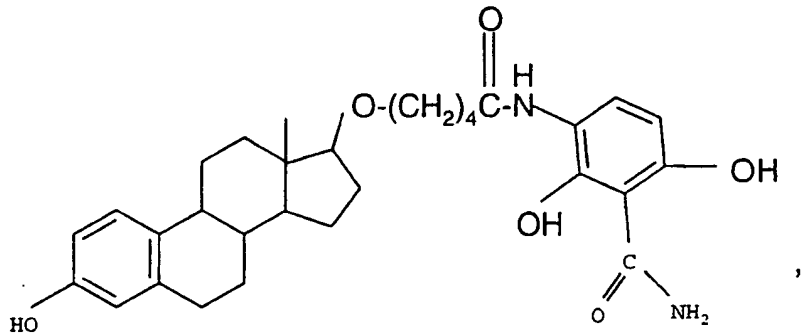












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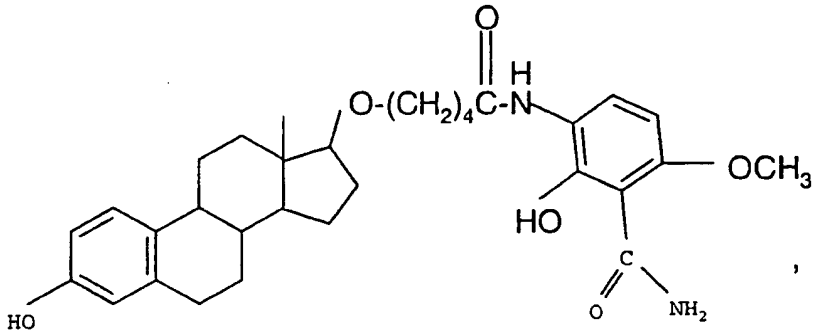

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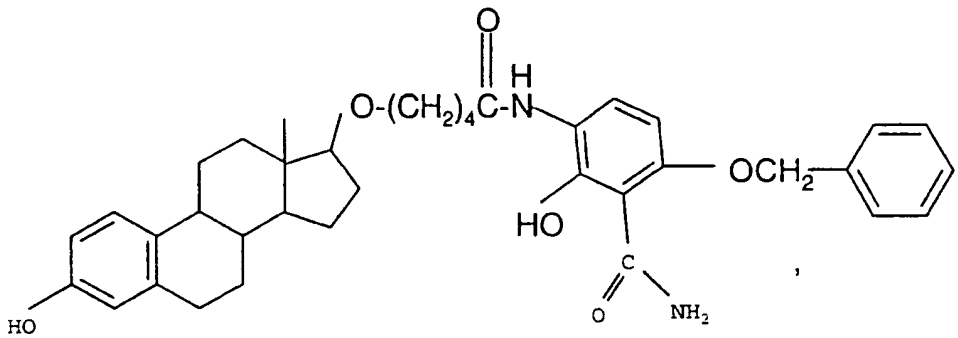

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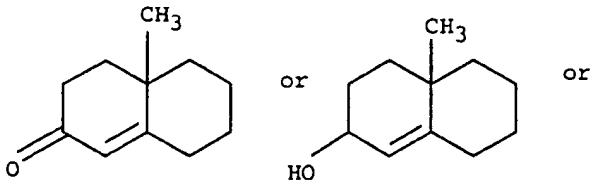
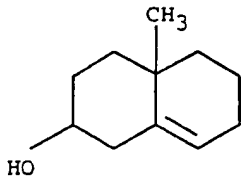
and the corresponding testosterone or 3-hydroxy-testosterone or DHEA derivatives, i.e., compounds of the above formula, except the A and B rings are



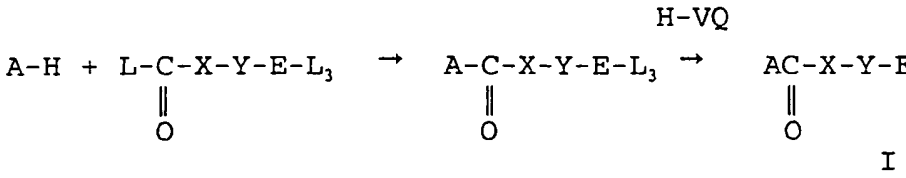




It is to be understood that all combinations and permutations of the various Markush groups for the different variables are contemplated to be within the scope of the present invention. In addition, the various stereoisomers generated therefrom are also contemplated to be within the scope of the present invention.

The compounds of the present invention are prepared by art recognized techniques. For example, the compounds of the present invention may be prepared by the schemes given hereinbelow. It is to be noted that the schemes depicted hereinbelow are exemplary and are applicable to any of the Q's contemplated in the present invention.

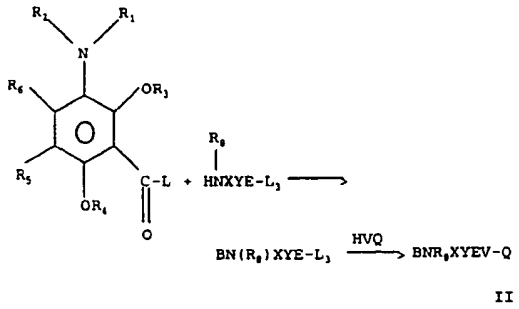
The reactants and reagents in the synthetic schemes depicted hereinbelow are commercially available or can be prepared by art recognized methods. For example, commercially available sex hormones (from which Q is derived) include estradiol, ethinyl estradiol, estrone, estriol, diethylstilbestrol, dienestrol, benzestrol, equilenin, testosterone and the like.

With respect to compounds of Formula I the following is exemplary.



I

while with compounds of Formula II, the following is exemplary



II

As defined herein AH, which contains an $NR_1$

group, reacts with L-C-X-Y-E-$L_3$ under amide forming conditions to form the corresponding amide, wherein L is

a leaving group, such as hydroxy, lower alkoxy, halogen, and the like and $L_3$ is hydrogen when Y-E ends with an NH or O, $L_3$ is halide or $OR_{21}$ wherein $R_{21}$ is hydrogen or lower alkyl when YE ends with an acyl group, or $L_3$ is halide, or brosylate, tosylate or mesylate when YE ends with a methylene ($CH_2$) group. The reaction may be conducted in a solvent which does not react with the reactants or products and in which the reactants are soluble. Preferably, the solvent is volatile. The reaction is conducted at effective temperatures. Preferably, the reaction is performed at room temperature up to the refluxing temperature of the solvent. The reaction is conducted for sufficient time to form the desired product.

The product thereof is $AC-X-Y-E-L_3$, which has at the

other end, i.e., the YE end, a functional group and which is reacted with the HV-Q under effective conditions. When YE ends with an acyl group and $L_3$ is a leaving group, such as halide or lower alkoxy, and V is O or NH then HV-Q reacts with $AC-X-Y-E-L_3$ under



acylating conditions. Alternatively, if HV is COOH and YE ends with an NH or O group, such that $L_3$ is hydrogen then the reaction of HVQ with $AC-X-Y-E-L_3$ is also

conducted under acylating conditions to form the compound of Formula I. For example, if QVH contains an hydroxy or amino, the hydroxy group or amino reacts with the acyl group on YE to form the ester or amide, respectively under acylating conditions. For instance, if E were a chemical bond, and Y were an acid or acylating derivative thereof, the reaction of HVQ with $AC(=O)-X-Y-E-L_3$ would form an ester or amide. On the other hand, HV may be carboxy which may optionally be converted to the corresponding acid halide; an amide or ester group is formed when the acylating derivative (acid or acid halide) reacts with the terminal OH or amino group on $YEL_3$ to form an ester or amide, respectively under acylating conditions.

The acylating reactions are preferably conducted in a solvent which does not readily react with either the products or the reactant and in which the reactants are readily soluble. Preferably, the solvent is volatile so that it can easily be removed by evaporation. The reaction is effected at effective temperatures which may range from room temperature up to the reflux temperature of the solvent and is conducted for sufficient time to form the desired product.

If, the linking group to Q is an ether, then VH is OH and YE ends with a methylene group and $L_3$ is a good leaving group such as halide or sulfonates, e.g., mesylate or aryl sulfonates, e.g., tosylate, brosylates,

and the like. Under those conditions, the reaction is conducted under Williams conditions, wherein QOH is converted to the corresponding $QO^{\ominus}$ with a strong base, such as sodium or potassium amide, and the product thereof is reacted with $ACXYEL_3$, wherein $L_3$ is halide

or other good leaving group, and YE ends with a $CH_2$ group. Alternatively, the QOH and

$ACXYEL_3$, wherein $L_3$ is halide may be reacted

directly with a hydroxide salt, such as KOH or NaOH, in dimethyl sulfoxide.

Alternatively, if QVH contains a hydroxy group at position 17 of the steroid and if XYE is

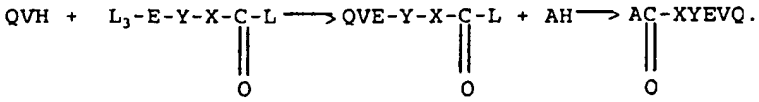
$C-(CH_2)_2-O$, QVH may be reacted with a cyclic anhydride, such as succinic anhydride, a mixed anhydride or an acyl halide under acylating conditions. The product of which reacts with AH to form the product of Formula I.

If on the other hand, VH is $NH_2$ and YE ends with a methylene group and $L_3$ is a good leaving group, such as halide or sulfonate or aryl sulfonate, the reaction between QVH and $ACXYEL_3$ form an amine linkage.

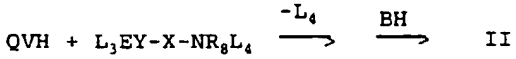
Alternatively, if Q contains an oxo group, such as at position 17 of the steroid and XYE contains a terminal amine function, Q may react directly with $AC\text{-}XYEL_3$ or $BNR_8\ XYEL_3$ wherein $L_3$ is hydrogen and more specifically the amine functionality present on YE, under reductive amination conditions to form the corresponding imine which is reduced catalytically, as for example by $H_2$lN or sodium cyanohydridoborate, to form an NH(V) linkage between Q and $XYE_{n1}$.

The reaction to form Formula II is similar to the reactions described hereinabove except that B contains the acyl group, so that BL is reacted with the amine $HR_8XYE\text{-}L_3$ under amide forming conditions to form $BN(R_8)XYE\text{-}L_3$, which in turn is reacted with $L_2VQ$ to form the product of Formula II. It is to be noted that $L_1$, $L_2$ and $L_3$, $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$, $R_8$, XYE, V, and Q are as defined hereinabove. Therefore, the comments hereinabove and hereinbelow with respect to the amide forming reactions and with the reactions with HQV are also applicable to these reactions.

Of course, the reactions may be performed in a reverse order. For example, the compound of Formula I may also be formed as follows:



Similarly, the compound of Formula II is formed by reacting:



wherein $L_4$ is hydrogen or if the amine is reactive with V or E, then it is an amine protecting group known in the art.

If any of the groups on A, B, X, Y, E, V, or Q have groups which are reactive with any of the reagents used or with any of the reactants or products, then they would be protected by protecting groups known in the art to avoid side reaction. These protecting groups normally used in synthetic organic chemistry are well known in the art. Examples are found in "PROTECTIVE GROUPS IN ORGANIC SYNTHESIS" by T.W. Greene, John Wiley & Sons, Inc., N.Y., 1981, ("Greene") the contents of which are incorporated by reference.

For example, if Q has OH group(s) that don't participate in the reactions described hereinabove, it is preferred that these OH groups be protected with hydroxy protecting groups known in the art prior to conducting those reactions. For example, the sex

hormones of Formulae IV, IV' or V or V' may have an OH group at C-3; it is protected by reacting with benzyl bromide to form the 3-benzyloxy derivative. After the desired reactions described hereinabove are completed, the protecting group is removed to afford the compounds of Formulae I and Formula II. For example, the 3-benzyoxy group can be converted to the 3-OH group by catalytic hydrogenation.

The various substituents described hereinabove on Q are substituted thereon using techniques known to the skilled artisan. The synthetic procedures for placing these substituents on the estrogens and androgens are known to the skilled artisan and can be accomplished utilizing known synthetic procedures.

The moieties substituted on the aromatic ring A and B are preferably effected by electrophilic aromatic reactions known in the art. To form the corresponding partially saturated or completely saturated ring, it is preferred that the aromatic ring is reduced. This can be effected by techniques known in the art, for example, catalytic hydrogenation.

With respect to compounds of Formulae IV or IV' and V and V', containing OH groups, substitutions can be effected on the A ring by electrophilic aromatic substitution and on the B, C, D rings by substitution reactions known in the art. With respect to compounds of Formula IV' and V', substitutions can be effected on the A, B, C, D ring by substitution reactions known in

the art. However, it should be noted that inasmuch as positions 9 and 6 of the estrogen steroid ring of the QVH moiety are more reactive than the other positions on the QVH moiety of Formula IV or V, if these positions contain a leaving group, these are more prone to substitution. This is especially true with respect to carbon-6 on the steroid ring. Thus, for example, if an hydroxy group is present on position 6 or position 9, reaction of steroidal QVH with N-bromosuccinimide will form the 6-Br and/or 9-Br-derivatives, which can be separated using techniques known to the skilled artisan. Reaction with either bromo derivative with ammonia followed by base will form the corresponding amine. Mono or dialkylation of the amine with lower alkyl halide will form the mono and dialkylamines respectively. Moreover, reaction of the 6-Br or 9-Br derivative formed hereinabove with a hydroxide base forms the corresponding alcohol. Moreover, reaction of the bromide with lower alkanol in the presence of a strong base, such as $OH^-$, under Williamson reaction conditions forms the corresponding lower alkoxy derivative.

Because of the increased activity, as described hereinabove, the 6 position of the steroid ring may be used as a link to YE.

An hydroxy group on C-11 is very reactive; oxidation of the OH with an oxidizing agent, such as

chromium oxide, yields the corresponding 11-oxo substituent.

If V is oxygen and Q-VH is a steroidal estrogen or androgen, it is preferred that the estrogen moiety has the structure of Formula IV and the androgen has the structure of Formula IV'. As indicated hereinabove, in the structure of Formulae IV and IV', the hydroxy group is substituted on the 17-position of the steroid. To facilitate substitution on the 16-position, after protection of the 3-hydroxy group, the 17-hydroxy group may be oxidized to a ketone using a standard oxidizing agent known in the art, e.g., acid dichromate, permanganate, ruthenium tetroxide, bromine, $MnO_2$ and the like. Once the ketone is formed, the C-16 becomes activated, facilitating substitution thereon. For example, alkylation at C-16 may be effected by converting the ketone to the 17-keto dimethyl hydrazone and then alkylating using n-butyl lithium as the base followed by an alkyl halide $R_{17}$ hal, wherein $R_{17}$ is lower alkyl and hal is halide. Hydrazone cleavage with cuprous chloride in aqueous tetrahydrofuran regenerates the C-17 ketones. Halogenation is effected by reacting the 17-ketone derivative with fluorinating agents, such as diethyl (2-chloro-1,1,2-trifluoroethyl) amine to form the 16-F derivative, or $Cuh_2$ where h is halide, e.g., chloro, bromo, to form the chloro and bromo derivatives, respectively. Reaction with Mercuric acetate followed by treatment with potassium iodide forms the 16-iodide.

Alkenylation and alkynylation can also be effected at C-16. These procedures are described in U.S. Patent No. 5,157,031 and 5,804,575 to Schwartz, et al., the contents of both of which are incorporated by reference.

The 16-hydroxy derivative can be prepared from the 16-halo derivative prepared as described hereinabove followed by reaction with hydroxide in aqueous solvent, e.g., pyridine as described, in the aforementioned patents. However, the 3, 16, 17, trihydroxy compound is known, i.e., estriol.

The amino derivatives are prepared by reactions of the 16-halo (e.g., bromo, chloro, or iodo derivatives) with ammonia followed by reaction with a strong base, such as hydroxide salt. Reaction of the amine with alkyl halide affords the alkyl amine. Further reaction with alkyl halide provides the dialkylamine.

After the substitutions are effected, then the protecting groups are removed at C-3. Reduction of the 17-keto by techniques known in the art, such as catalytic hydrogenation, affords the 17-hydroxy derivative, which is then free to react, as described hereinabove, to form the compounds of Formula I and II.

Alternatively, for estrogen derivatives, estriol may be used as the starting material. Both the 3-OH and 17-OH groups are protected by protecting groups such as those described in Greene, supra, and then the hydroxy group at carbon 16 may be converted to a good

leaving group, such as halide, tosylate or mesylate, and then the desired derivative at carbon-16 may be effected by substitution reactions known in the art. For example, if an amine is desired, the tosylate or other leaving group at C-16 is reacted with ammonia followed by base, such as hydroxide salt to form the corresponding amine. To form the corresponding monoalkyl or dialkyl amine, the amine is reacted with alkyl halide using techniques known to the skilled artisan. If halo is desired, then the tosylate or other leaving group at C-16 is reacted with the appropriate halide, H-hal, e.g., in aqueous solvent, or $Phal_3$ and the like, wherein hal is halide. If the ether is desired, then the hydroxy group is converted to the corresponding base and then reacted with alkyl halide under Williamson synthesis conditions.

If the hydroxy group is desired at the 16-position with only hydrogens at the 17-position, then the 17-keto derivative formed hereinabove may be converted to methylene using techniques known in the art, e.g., zinc amalgam and aqueous HCl under Clemmensen reduction procedure; hydrazine hydrate and base, such as NaOH or KOH under Wolff Kishner reduction conditions, especially in refluxing diethylene glycol. (Huang-Minlon modification of the Wolff-Kishner reaction). Alternatively, the reduction can be carried out in dimethyl sulfoxide with potassium t-butoxide as the base.

It should be noted that when there is a hydroxy, amino or carboxy group at C-16, then attachment to the main chain may be effected by bonding through the oxygen, nitrogen or (CO) or C-NH respectively at the

16-position, as depicted in Formulae V or V'. For example, if V is O, as indicated hereinabove, the $C_{16}$ hydroxy group is prepared by reacting the 17-keto derivative, e.g., estrone, with CuBr, followed by reaction with hydroxide base. If further substitution is effected on the estrogen, the C-16 hydroxy group is protected with a protecting group known in the art, as described in Greene, supra. If substitution is to occur on the 17 position, then the 17-keto is reduced to the hydroxy group using reducing agents known in the art, e.g., hydrogenation catalysts. It may then be converted to the tosylate or mesylate or brosylate derivative, and then the other substituents at C-17 defined by $R_{18}$ can be placed on the steroid ring by standard substitution reactions with the appropriate reagents, as described hereinabove. For example, an amine may be formed by reacting the toxylate with am amide salt such as $NaNH_2$, while the mono and dialkylamine may be formed therefrom by reacting the amine with alkyl halide. Halo groups may be placed on the 17-position by reacting the tosylate with halide salt. Alkyl groups may be substituted on C-17 by reacting the halide or tosylate salts with an organo metallic reagent, such as the Gilman reagent.

Alternatively, if there is an amino group on C-16, then attachment to the main chain may be effected by reacting the amino group with a carboxy group on YE to form the corresponding amide. The amino group can be formed from the 16-hydroxy derivative by techniques known in the art. For example, the 16-$NH_2$ group is prepared by reacting the 17-keto derivative, e.g., estrone, with CuBr, followed by reaction with an amide salt, -$NH_2$, such as sodium or potassium amide.

The 3-O-phosphate derivatives of the sex hormones can be formed by carbodiimide catalyzed condensation of the estrogen or androgen having a hydroxy group on C-3 with o-phosphoric acid under effective coupling conditions. It is preferred that, if the 3-O-phosphate is present, that it be the last substituent added to the steroidal ring, and it is more preferred that it is the last reaction in the synthetic scheme.

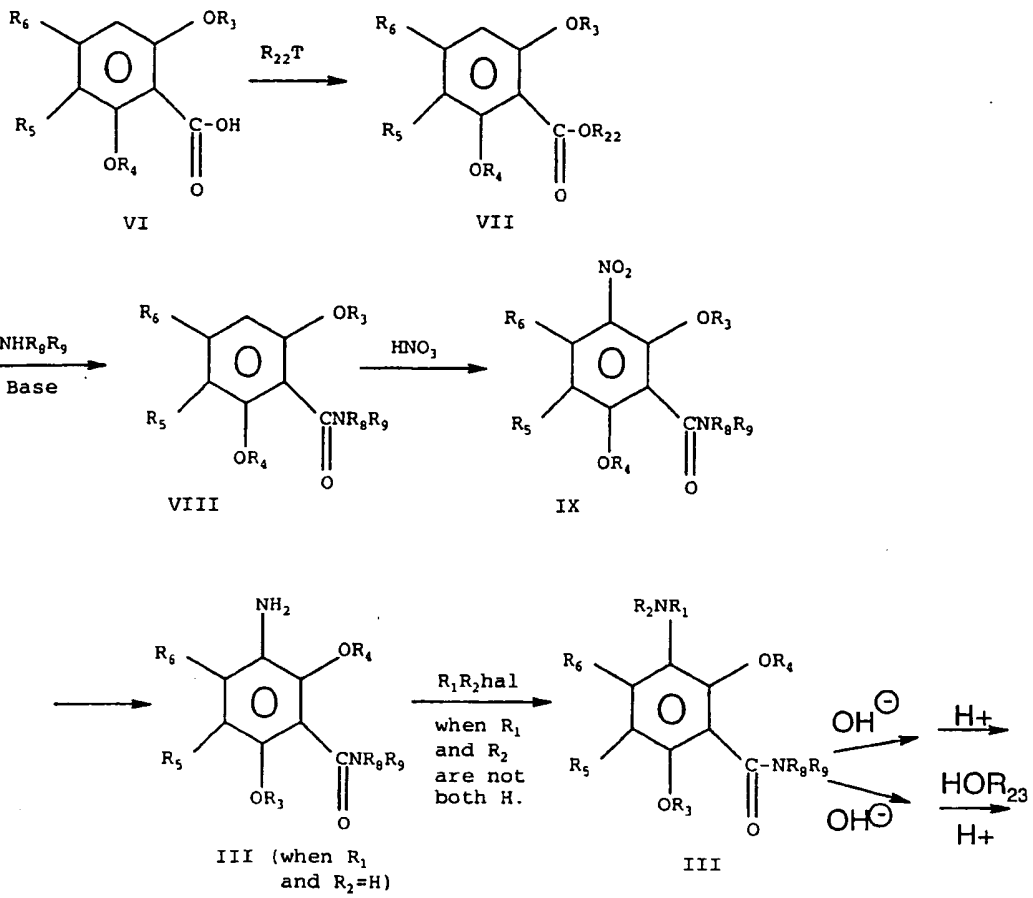
Although not necessarily present in the estrogens, in the androgen, the C-3 position may be substituted with oxo. If the androgen has, for example, an oxo substituent at C-3, such as testosterone, the oxo groups may be reduced by reducing agents known in the art, such as lithium aluminum hydride, sodium borohydride, and the like. The resulting 3-hydroxy substituent may be the desired substituent at the 3 position. Alternatively, it then may be used to form the 3-phosphate derivative, as described hereinabove. On the other hand, if the androgen has a 3-hydroxy

substituent and the desired product is 3-oxo, then the 3-hydroxy group can be converted to the corresponding ketone utilizing standard oxidizing agents known in the art, e.g., acid dichromate, permanganate, ruthenium tetroxide, bromine, mono and the like.

These synthetic routes described hereinabove are exemplary. After the appropriate groups are substituted on the estrogen rings, then the protecting groups if any, are removed, as in the example hereinabove, wherein in the last step, the protecting group is removed at C-16.

It should be noted that there is a relationship between the compounds of Formula III and A and B. More specifically, A and B are derivatives of the compound of Formula III, that is, A is the compound of Formula III less a $R_2$ group, while B is the compound of Formula III less the $R_7$ group. If the compound of Formula I is to be formed, then the definitions of $R_1$, $R_3$, $R_4$, $R_5$, $R_6$ and $R_7$ are as defined above, but $R_2$ is hydrogen. On the other hand, if the compound of Formula II is formed, then $R_1$, $R_2$, $R_3$, $R_4$, $R_5$ and $R_6$ are as defined hereinabove and $R_7$ is hydroxy or lower alkoxy.

The compound of Formula III is prepared by art recognized methods known to the person skilled in the art. For example, the following scheme is exemplary.




wherein $R_1$, $R_2$, $R_3$, $R_4$, $R_5$, $R_6$, $R_7$, $R_8$, $R_9$ are as defined hereinabove and $R_{22}$ is lower alkyl or aryl and hal is halide (e.g., Br, I, or Cl) and T is hal, especially bromides and iodides. The corresponding saturated or

partially saturated compounds of Formula III may be formed by catalytic hydrogenation.

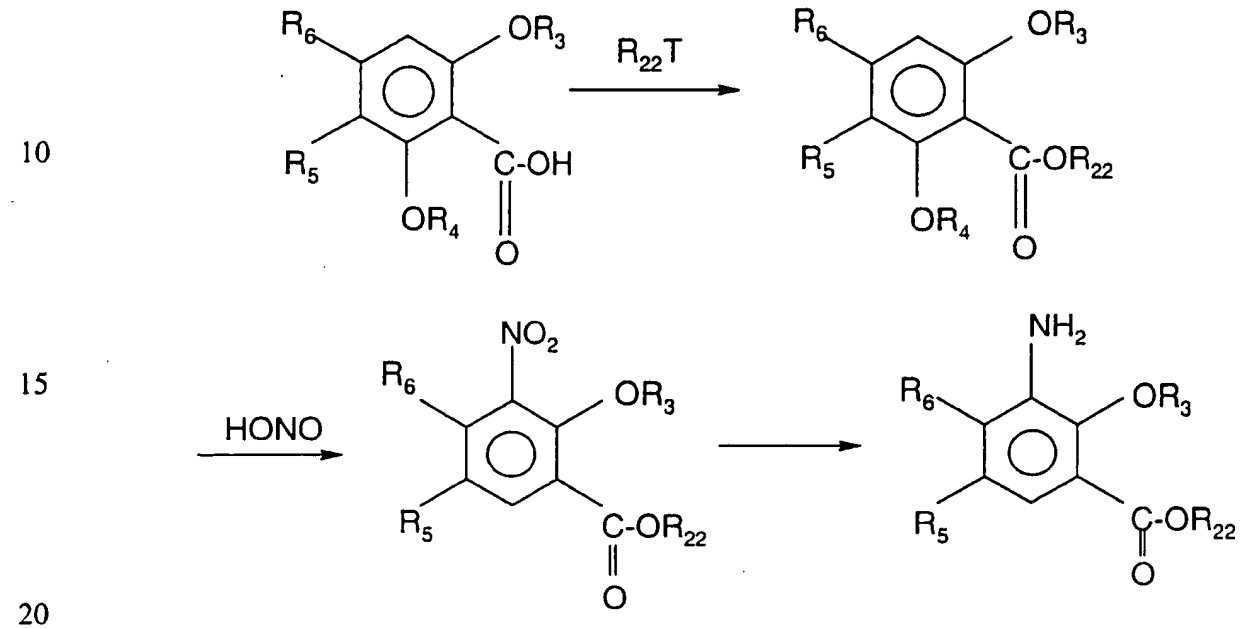
The synthesis illustrated hereinabove to form the compound of Formula III is exemplary. The starting material for this synthesis is either commercially available or is prepared easily from a commercially available material.

The objective to form the compound of Formula VIII from the compound of Formula VI is to convert the acid functionality to the corresponding amide. If neither $R_3$ nor $R_4$ are hydrogen, then one method is to convert the acid to the corresponding acyl halides utilizing halogenating reagents, such as thionyl chloride, $PM_3$, $PM_5$, (wherein M is Cl or Br), $Ph_3P$ in $CCl_4$, cyanuric fluoride, and the like, and the acid chloride is reacted with $NHR_8R_9$ to form the corresponding amide. However, if either $R_3$ or $R_4$ is hydrogen, the hydroxy group is reactive with many of these reagents, e.g., $SOCl_2$, $PM_3$ and $PM_5$, and this route cannot be taken. In this case, the hydroxy group may be protected using protecting groups described in Greene, supra, the contents of which are incorporated by reference, such as converting the alcohol to methoxymethyl (MOM) or 2-methoxyethoxymethyl (MEM) and then the protecting group is removed at the end of the reaction.

Alternatively, as shown in the scheme hereinabove, the acid functionality is converted to an ester under Fischer esterification conditions, which is

then reacted with the amine to form the amide. In the method illustrated, the carboxylic acid VI is reacted with a base, such as hydroxide and then the corresponding salt is reacted with an alkyl halide ($R_{22}$hal), wherein $R_{22}$ is a lower alkyl and hal is halide, especially bromides and iodides, to form the corresponding ester, which in turn is reacted with the amine $NHR_8R_9$ in base (such as hydroxide) to form the corresponding amide (VIII). This product in turn is reacted with nitric acid to form the corresponding nitro compound IX, which is reduced by reducing agents known in the art, such as Zn, Sn or Fe and acid, or Pd/C and the like to form the primary amine, i.e., the compound of Formula III when $R_1$ and $R_2$ are both hydrogen. This product in turn may be reacted with $R_1R_2$hal if an alkylamine or dialkylamine is desired. The product formed is a compound of Formula III wherein $R_7$ is $NR_8R_9$. If a acid is desired (that is if $R_7$ is OH), then the amide is hydrolyzed under effective hydrolyzing conditions, such as by reacting the amide in an inert organic solvent in the presence of hydroxide base and heating at effective temperatures, such as, for example at a temperature ranging from room temperature until the reflux temperature of the solvent and then adding acid in work-up. Alternatively, an ester may be formed by hydrolyzing the amide product in III, and reacting the product with $R_{23}$OH, wherein $R_{23}$ is lower alkyl in the presence of an acid catalyst, such a p-toluene sulfonic

acid or a mineral acid such as HCl, $H_2SO_4$, and the like, under esterification conditions.

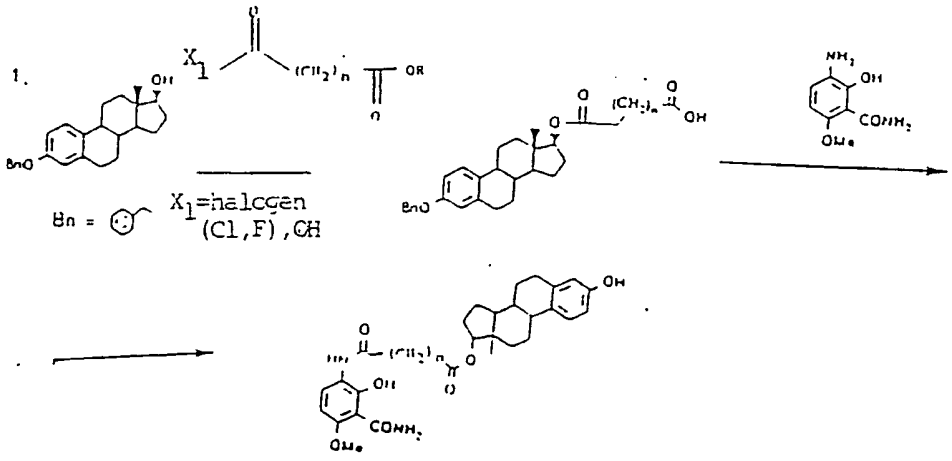


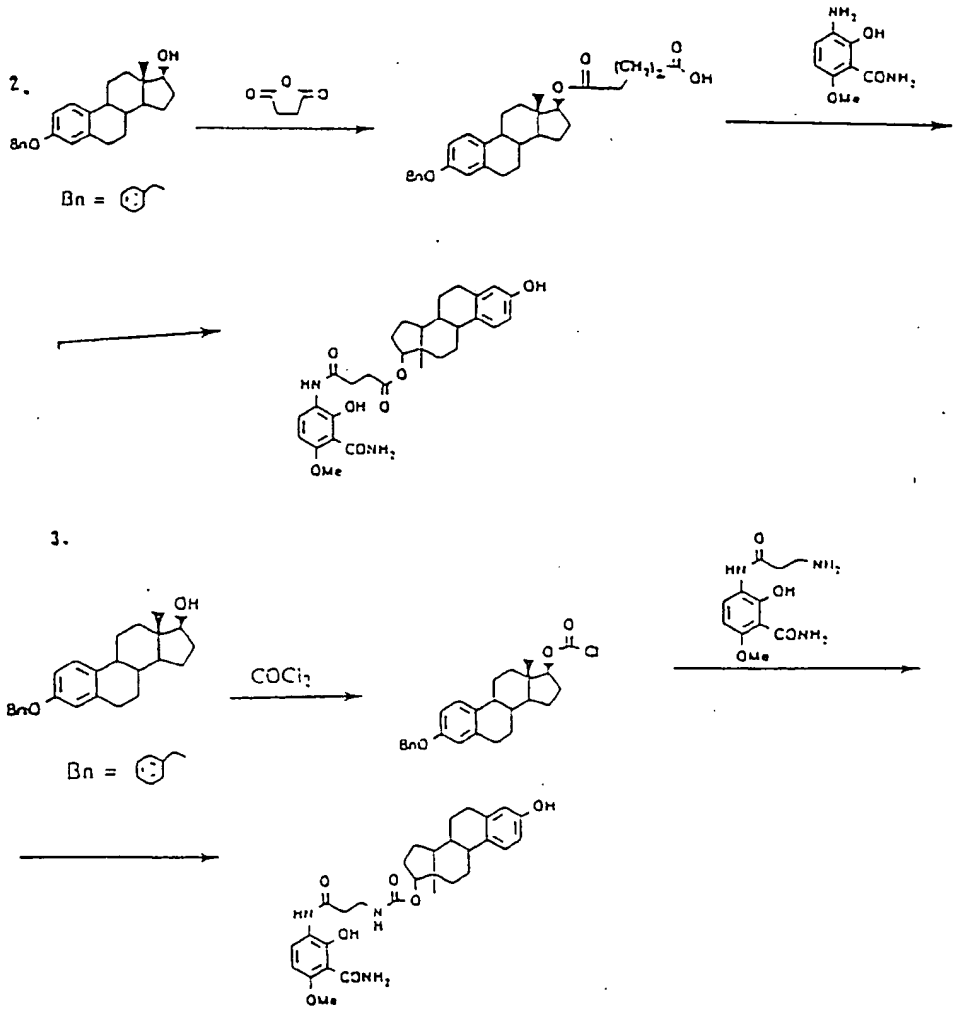
Alternatively, the amine (Compound III) may be formed by reacting the product of VII with nitrous acid as described above and the nitro group is reduced under conditions described hereinabove to form the corresponding amine. It should be noted that in this synthesis, if $R_7$ is lower alkoxy, then $R_7$ is the same as $OR_{22}$.

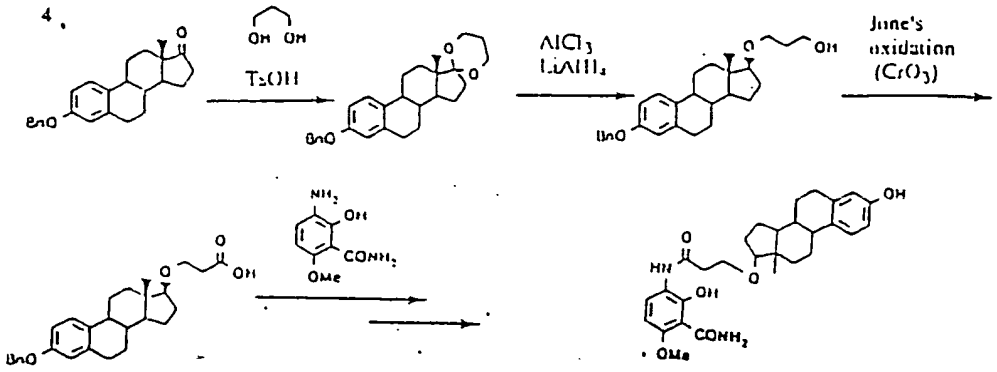
It is to be noted that the examples, e.g., Ex. 1 and 2 exemplify various procedures for preparing the compound of Formula III.

Again as with the synthesis of compounds of Formula I and II, protecting groups may be used if any of the groups of $R_5$, $R_6$, $R_4$, $R_3$ or $R_7$ were reactive with any of the reagents used or with any of the products. Again, these protecting groups are known in the art. Examples are found in Greene, <u>supra</u>, the contents of which are incorporated by reference.

Exemplary procedures to illustrate the above schemes are shown hereinbelow:




2.
Bn =
3.
Bn =
COCl2




As described hereinabove, the compounds of the present invention are particularly characterized by two active moieties. One moiety, the cyclic amide portion of the molecule, as defined herein, exhibits bone seeking affinity. In this context, the bone seeking affinity is defined as having the capability to bind to calcium with a tendency to accumulate in bone and to incorporate into its crystal lattice. The inventors have found that one of the intermediates in the process of preparing the compounds of the present invention, the cyclic amide, e.g., the benzamide compound of Formula III, exhibits bone-seeking affinity, and is also contemplated to be another aspect of the present invention.

The second necessary constituent of the present compound has a bone active moiety which interacts with the bone and affects bone metabolism by

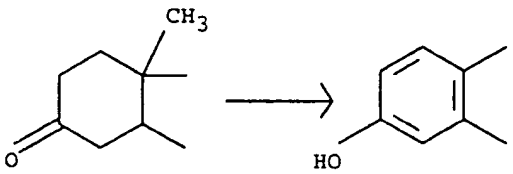
inhibiting bone resorption and/or increasing bone formation or both. The second component is as defined hereinabove.

The efficacy of the compounds of the present invention can be facilitated immediately at the bone site, having the active site of both the cyclic amide and the bone active moieties available (not a pro-drug). Preferably, however, the efficacy of the compounds of the present invention can be facilitated stepwise, first by the bone-seeking moiety which affinity localizes the compound at the bone site. Once anchored at the bone site, the other moiety of the molecule, i.e., the bone active domain, interacts with the bone and effects either an increase in bone formation or inhibits bone resorption. On the other hand, the compounds of the present invention may be pro-drugs, i.e., the active site regarding bone activity is internal in the compound and not immediately available and will exhibit no initial activity. However, when subjected to the enzymatic hydrolytic conditions occurring at the bone site, the bone active domain will be released. This action reflects an idealized feed back system and is elicited only in response to the specific need.

The bone targeted androgens, e.g., testosterone or androstenedione, serve as precursors. Without wishing to be bound, it is believed that they may be metabolically activated by aromatase to estrogen in vivo. More specifically, the A ring of the androgen

testosterone were the androgen utilized in the compounds of the present invention of Formula I or II, herein,

when subjected to aromatase enzyme <u>in vivo</u> at the bone targeting site, the A ring




A ring of testosterone A ring of estrogen

of testosterone is converted to the A ring of estrogen. Without wishing to be bound, it is believed that the metabolic enzyme system responsible for the transformation is present in, <u>inter alia</u>, the bone; thus when the bone targeting agent is anchored to the bone, as depicted hereinabove, the aromatase enzyme can react with the anchored androgen moiety and convert it to the corresponding estrogen. Thus, both the androgen, which is administered to the patient and the resulting estrogen product, are contemplated by the present invention.

The present new compounds may be present in their pharmaceutically acceptable salts, i.e., salts which are safe and non-toxic to the mammal. If the compounds contain basic nitrogen, salts can be formed

with either inorganic or organic acids. All such acid salts are contemplated by the invention, but especially preferred are salts with pharmaceutically acceptable acids, such as hydrochloric, sulfuric, nitric, toluene sulfonic, acetic, propionic, tartaric, malic and similar such acids well known in the art. In addition, quaternary salts can be formed using standard techniques of alkylation employing, for example, hydrocarbyl halides, or sulfates, such as methyl, ethyl, benzyl, propyl or alkyl halide or salts. On the other hand, acid functionalities present on the compounds including, the o-phosphoric acid functionality, ($OPO_3H$) can form salts with acids. The pharmaceutically acceptable salts in this case are non-toxic and include the alkali metal salts, such as sodium, potassium, lithium or the alkaline earth metal salts, such as calcium or magnesium as well as aluminum, zinc and the like.

The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in optically active forms. The various in stereoisomeric forms are contemplated to be within the scope of the present invention. Moreover, the compounds of the present invention can be mixtures of the isomers, including racemic mixtures which are also contemplated to be within the scope of the present invention. Depending upon the substituents, the present compounds may form addition salts as well. All of these other

may form addition salts as well. All of these other forms are contemplated to be within the scope of the present invention.

The active ingredients of the therapeutic compositions are the compounds of the present invention. They are useful for treating and/or preventing degenerative bone disorders when administered in effective amounts. These amounts can be determined by a physician. However, it is preferred that the active ingredients be administered in amounts ranging from about 0.1 μg to about 100 mg per kilogram of body weight per day. A preferred dosage regimen for optimum results ranges from about 1 μg to about 10 mg per kilogram of body weight per day, and such dosage units are employed so that a total of from about 7 μg to about 700 mg of the active compound for a subject of about 70 kg of body weight are administered in a 24-hour period. This dosage regimen may be adjusted to provide the optimum therapeutic response and is preferably administered once a day in dosages of about 50 mg per administration. For example, several divided doses may be administered daily or the dose may be proportionally reduced as indicated by the exigencies of the therapeutic situation. A decided practical advantage is that the active compound may be administered in a convenient manner such as by the oral, intravenous, intramuscular or subcutaneous routes.

The active compound may be orally administered, for example, with an inert diluent or with an assimilable edible carrier, or it may be enclosed in hard or soft shell gelatin capsule, or it may be compressed into tablets, or it may be incorporated directly with the food of the diet. For oral therapeutic administration, the active compound may be incorporated with excipient and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 5% of active compound. The percentage of the compositions and preparations may, of course, be varied and may conveniently be between about .01 to about 10% of the weight of the unit. The amount of active compound in such therapeutically useful compositions is such that a suitable dosage will be obtained. Preferred compositions or preparations according to the present invention are prepared so that an oral dosage unit form contains between about 5 and 500 mg of active compound.

The tablets, troches, pills, capsules and the like may also contain the following: A binder such as gum tragacanth, acacia, corn starch or gelatin; excipient; disintegrating agents such as corn starch, potato starch, alginic acid and the like; lubricants; and a sweetening agent such as sucrose, lactose or saccharin may be added or a flavoring agent such as peppermint, oil of wintergreen, or cherry flavoring.

When the dosage unit form is a capsule, it may contain, in addition to materials of the type, a liquid carrier. Various other materials may be present as coatings or to otherwise modify the physical form of the dosage unit. For instance, tablets, pills, or capsules may be coated with shellac, sugar or both. A syrup or elixir may contain the active compound, sucrose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any dosage unit form should be pharmaceutically pure and substantially non-toxic in the amounts employed. In addition, the active compound may be incorporated into sustained-release preparations and formulations.

The active compound may also be administered parenterally or intraperitoneally. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of

manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents, delaying absorption, for example, aluminum monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other

ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze-drying technique which yield a powder of the active ingredient plus any additional desired ingredient from previously sterile-filtered solution thereof.

As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active ingredient, its use in the therapeutic compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions.

It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the novel dosage unit forms of the invention are

dictated by and directly dependent on (a) the unique characteristics of the active material and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active material for the treatment of disease in living subjects having a diseased condition in which bodily health is impaired as herein disclosed in detail.

The principle active ingredient is compounded for convenient and effective administration in effective amounts with a suitable pharmaceutically acceptable carrier in dosage unit form as hereinbefore disclosed. A unit dosage form can, for example, contain the principle active compound in amounts ranging from about 0.1 to about 1000 mg, with from about 5 to about 500 mg being preferred. Expressed in proportions, the active compound is generally present in from about 1 to about 100 mg/ml of carrier. In the case of compositions containing supplementary active ingredients, the dosages are determined by reference to the usual dose and manner of a administration of the said ingredients.

As used herein, the bridging group is the group $\overset{O}{\overset{\|}{C}}XYEV$ or $NR_8XYEV$, that, is the group that links A or B, respectively to the bond active domain. When the term, the "linkage of Q to the bridging group" or equivalent, is used herein, this refers to the linkage of Q to the bridging group or any part of the bridging

group. For example, it refers to the EV linkage, but if E is a chemical then the YEV linkage or if Y and E are both chemical bonds, the XYEV linkage.

Unless indicated to the contrary, all percent are by weight.

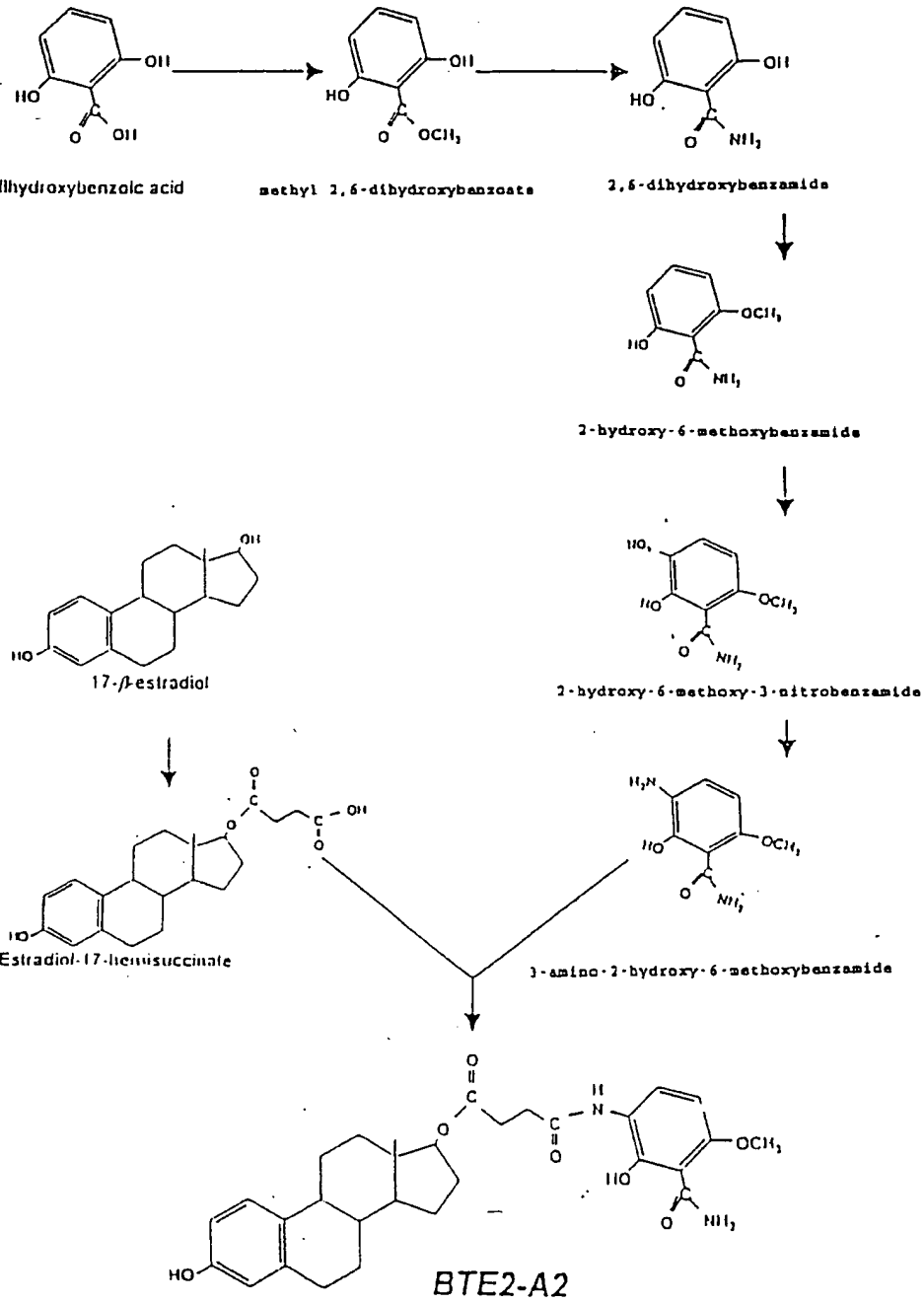
The plural refers to the singular, and vice versa.

The following non-limiting examples further illustrate the present invention.

## EXAMPLE 1

The following scheme depicts the synthetic route utilized to prepare the product described in Example 1.

**Synthesis of BTE2-A2**



**BTE2-A2**

**A. 2,6-Dihydroxybenzoic acid**

2,6-Dihydroxybenzoic acid (CAS [303-07-1] was purchased from Aldrich Chemical Company and was recrystallized from hot water then dried in a vacuum oven before use.

**B. Methyl 2,6-Dihydroxybenzoate**

One hundred grams (0.65 mol) of 2,6-dihydroxybenzoic acid was dissolved in 1000 mL of $NH_4OH$ (28% ammonia) and 1.1 eq of $AgNO_3$ was added. 1.1 eq of $CH_3I$ was added over 30 minutes at 0-5°C. The mixture was allowed to stir for 12 hours. Methyl iodide and ammonia were removed under reduced pressure and sample was recovered by filtration. Yield was 91.74 g.

**C. 2,6-Dihydroxybenzamide**

One hundred grams of methyl 2,6-dihydroxybenzoate (0.595 mol) was dissolved in 1000 mL of $NH_4OH$ (28% $NH_3$) and stirred occasionally at room temperature for 24 hours. Ammonia was removed under reduced pressure and the product crystallized and was recovered by filtration. The overall yield was 78.5%. The residual water was removed by dissolving in benzene and removing water by azeotropic distillation using a

Dean-Stark trap. Benzene was removed under reduced pressure.

$^1$H NMR (500 MHZ, DMSO-$d_6$) δ 12.64 (s, 2H, 2-OH and 6-OH), 8.20 (d, 2H, $NH_2$), 7.16 (t, 1H, J=8.5 Hz, H4, H3 or H5), 6.35 (d, 2H, H3 and H5, J=8.5 Hz, H3 or H5, H4).

$^{13}$C NMR (500 MHZ, DMSO-$d_6$) δ 172.37 (C=0), 160.70 (C2 and/or C6), 133.63 (C3 and/or C5), 107.09 (C4), 102.39 (C1). DMSO peak calibrated at δ 39.5.

**D. 2-hydroxy-6-methoxybenzamide**

In a 500 mL round-bottomed flask 16.5 g (0.1078 mol) of 2,6-dihydroxybenzamide was dissolved in 300 mL of dry acetone. To this was added 32.79 g (0.2372 mol) $K_2CO_3$ and the resultant product was stirred for 30 minutes at room temperature. $(CH_3)_2SO_4$ (12.24 ml/16.32 g/0.1293 mol) was added dropwise and the reaction mixture was heated to reflux and held there for 15 h. The mixture was cooled in an ice bath and then was filtered.

The filtered solid was washed with 3 x 50 mL portions of acetone. Acetone was then removed using a rotary evaporator. The residue was mixed with 1N NaOH, then 300 mL $CHCl_3$ was added and the mixture was transferred to a separatory funnel. The lower layer was removed and the aqueous phase was further washed with 2 x 50 mL portions of $CHCl_3$.

The aqueous layer was acidified to pH=3-4 with 1N HCl and a white precipitate formed. The precipitate was redissolved in 300 mL $CHCl_3$, then washed with 2 x 100 mL portions of 1 M NaCl (aq). The chloroform extract was concentrated under rotary evaporation. Finally traces of water and $CHCl_3$ were removed by azeotropic distillation from a benzene solution. Benzene was stripped under vacuum distillation. Yield = 13 gms (72.22%).

**E. 2-hydroxy-6-methoxy-3-nitrobenzamide**

2-hydroxy-6-methoxybenzamide (8.37 g/0.05 mol) was dissolved in 100 mL of glacial acetic acid in a 500 mL round bottom flask. This solution was cooled in an ice bath and concentrated $HNO_3$ (10 mL/0.157 mol) was added in three portions over a period of 30 minutes. The mixture was stirred for 6 hours at 0-5°C then for an additional 18 hours at room temperature.

The reaction mixture was diluted with 1.0 L of cold water and the resulting solid was recovered by filtration. The solid was washed with 500 mL of $H_2O$ then 3 x 100 mL portions of ethanol. The product was dried in a vacuum desiccator over $P_2O_5$. Product mass was 7.3 g (68.7%).

$^1H$ NMR (500 MHZ, DMSO-$d_6$) δ 15.72 (s, 1H, 2-O<u>H</u>), 8.42 (d, 2H, C(=O)-N<u>H</u>$_2$), 8.15 (d, 1H, H4, J=8.5Hz, H4, H5),6.74

(d, 1H, H5, J=8.5 Hz, H5, H4), 4.00 (s, 3H, $OC\underline{H}_3$).

$^{13}C$ NMR (500 MHZ, DMSO-$d_6$) δ 170.44 (C=0), 163.04 (C3), 158.90 (C2), 131.53 (C1), 130.71 (C4), 105.25(C6), 101.86 (C5), 57.14 ($-O\underline{C}H_3$).

**F. Synthesis of 3-amino-2-hydroxy-6-methoxybenzamide**

7.3 g of 2-hydroxy-6-methoxy-3-nitrobenzamide was slurried in 250 mL of dry $CH_3OH$, and 2.5 g of 10% Pd/C catalyst was added. The mixture was placed in a Parr hydrogenator under 46 p.s.i. of $H_2$. The mixture was held for 18 hours during which time the $H_2$ pressure decreased to 9.5 p.s.i.

The mixture was filtered using Celite and concentrated under vacuum at 35 - 40°. The yield was 5.5 g (87.9%).

$^1H$ NMR (500 MHZ, DMSO-$d_6$) δ 14.43 (s, 1H, 2-$O\underline{H}$), 8.15 (d, 2H, $-C(=O)-N\underline{H}_2$), 6.73 (d, 1H, H5, J=8.5 Hz, H5, H4), 6.33 (d, 1H, H4, J=8.5 Hz, H4, H5), 4.38 (s, 2H, 5-$N\underline{H}_2$), 3.79 (S, 3H, $-OC\underline{H}_3$).

$^{13}$CNMR (500 MHZ, DMSO-$d_6$) δ 172.41 (C=0), 151.31 (C2), 149.68 (C3), 131.32 (C6), 116.57 (C5), 102.58 (C1), 100.64 (C4), 55.96 (-O<u>C</u>H$_3$).

**G. Synthesis of estradiol-17-hemisuccinate**

Ten grams of 17-β-estradiol was dissolved in 200 mL benzene with 4 mL pyridine in a round-bottom flask fitted with a Dean-Stark trap. The solution was heated to relux to remove traces of water. The solution was cooled and 12 g (excess) of succinic anhydride was added. The mixture was heated to reflux and held for 24 h.

The mixture was cooled and filtered, then concentrated using rotary evaporation. Fifty mL $CH_3OH$ was added, along with 2 g $NaHCO_3$ and 10 mL $H_2O$. The mixture was stirred for 12 h and filtered. To the filtrate 1 N HCl was added until pH=7, then 2.0 L of 0.1 N HCl (cold) was added. The product was recovered by filtration and was recrystallized from benzene. 12 g of product were recovered.

**H. Synthesis of BTE2-A2**

Four grams of estradiol-17-O-hemisuccinate was dissolved in 25 mL dimethylformanide (DMF) and 1.4512 g of N-hydroxybenzotriazole (HOBT) was added. The mixture was cooled to 0-5°C and was stirred for 30 minutes.

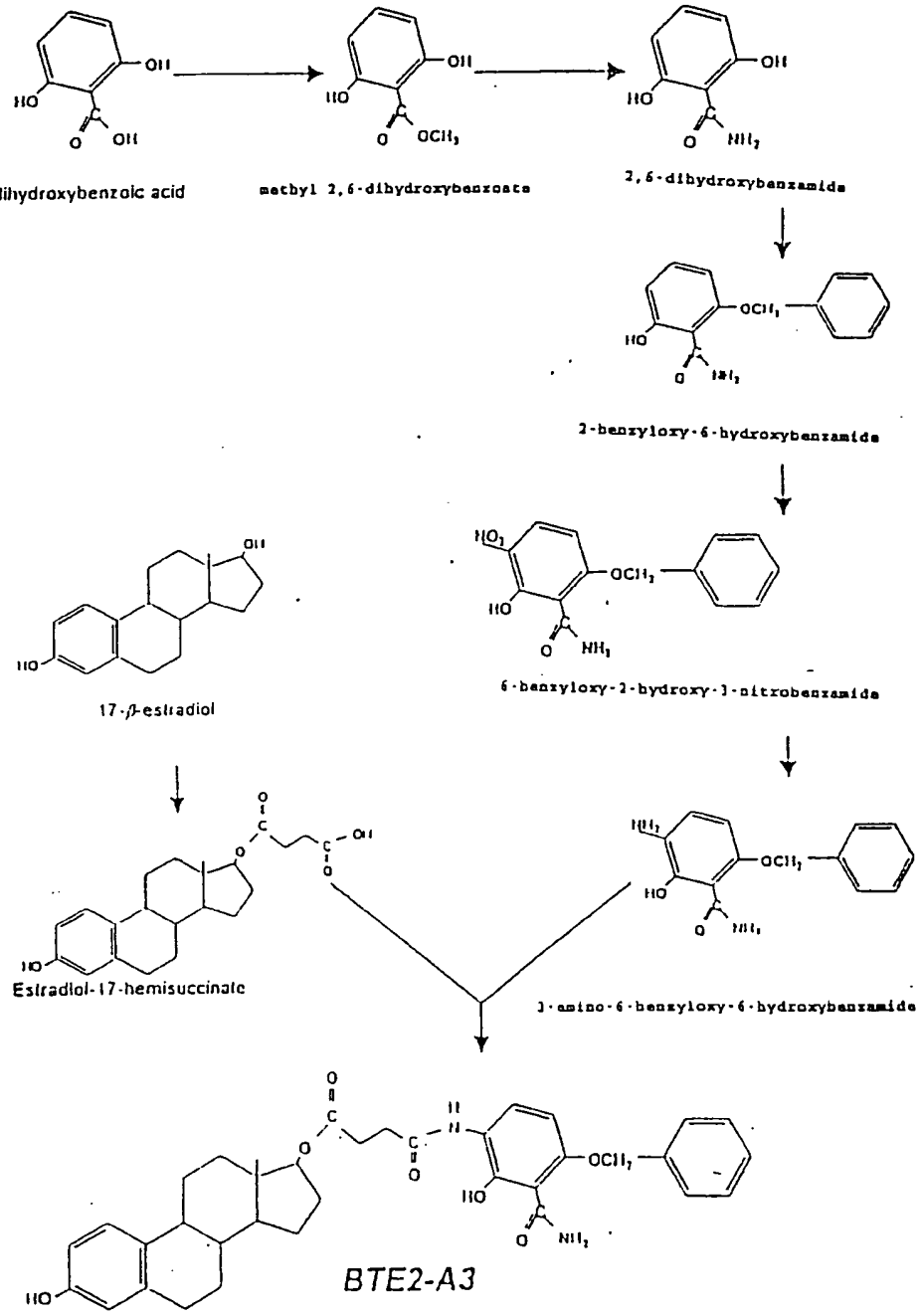
Diisopropylcarbodiimide (1.34 g) was added, followed by stirring 30 minutes at 0-5°C. A solution of 1.955 g of 3-amino-2-hydroxy-6-methoxy-benzamide in 10 mL DMF was added and the mixture was stirred for 24 hours at room temperature. The reaction was stopped by addition of 200 mL $H_2O$ and the mixture was extracted with 3 x 100 mL portions of ethyl acetate which was then washed with $H_2O$, then 2 x 25 mL portions of 10% aqueous $NaHCO_3$, and then with $H_2O$ again. The ethyl acetate solution was dried over $Na_2SO_4$ and concentrated under reduced pressure. Further purification was by silica gel chromatography, developed with ethyl acetate:n-hexane, 1:1, v/v. Yield=87%. $R_f$=0.25.

$^1H$ NMR(500 MHZ, $CDCl_3$) δ 14.46 (s, 1H, 25-O<u>H</u> ), 8.37 (d, 1H, H29, J=9.00 Hz, H29, H28), 8.03 (d, 2H, 30-C(=O)-N<u>H</u>$_2$), 7.08 (d, 1H, H2 ,J=8.5 Hz, H2, H1), 6.60 (d, 1H, H1, J=8.5 Hz, H1, H2), 6.53 (s, 1H, H4), 6.36 (d, 1H, H28, J=9.00 Hz, H28, H29), 5.97 (s, 1H, 23-N<u>H</u>), 5.56 (s, 1H, 3-O<u>H</u>), 4.69 (t, 1H, H17), 3.90 (s, 3H, 31-OC<u>H</u>$_3$), 2.77-2.70 (m, 6H, C<u>H</u>$_2$-6, C<u>H</u>$_2$-20, C<u>H</u>$_2$-21), 2.18-1.22 (m, 10H, remaining $CH_2$, ie 7, 15, 16, 11, 12), 0.76 (s, 3H, 18-C<u>H</u>$_3$).

**EXAMPLE 2**

The following scheme depicts the synthetic route described in the following example.

*Synthesis of BTE2-A3*



**A. 2,6-Dihydroxybenzoic acid**

2,6-Dihydroxybenzoic acid (CAS[303-07-1]) was purchased from Aldrich Chemical Company and was recrystallized from hot water then dried in a vacuum oven before use.

**B. Methyl 2,6-Dihydroxybenzoate**

This was prepared in accordance with the procedure in Example 1.

**C. 2,6-Dihydroxybenzamide**

This was prepared in accordance with the procedure in Example 1.

**D. 6-benzyloxy-2-hydroxybenzamide**

In a 500 mL round-bottomed flask 24g of 2,6-dihydroxybenzamide was dissolved in 500 mL of dry acetone. To this was added 50 g $K_2CO_3$ and the resultant product was stirred for 30 minutes at room temperature. Benzyl bromide (1.2 eq.) was added dropwise and the reaction mixture was heated to reflux and held there for 15 h. The mixture was cooled in an ice bath and then was filtered.

The filtered solid was washed with 3 x 50 mL portion of acetone. Acetone was then partially removed using a rotary evaporator. The product was filtered and washed with water then recrystallized from benzene, yield = 84%. mp = 179°-181°C.

$^1$HNMR (500 MHZ, DMSO-$d_6$) δ 14.01 (s, 1H, 2-OH), 8.13 (d, 2H, $NH_2$), 8.16-7.34 (m, 5H, $C_6H_5$), 7.30 (t, 1H, H4, J=8.5 Hz, H4, H3 and/or H5), 6.63 (d, 1H, H5, J=8.5 Hz, H5, H4), 6.50 (d, 1H, H5, J=8.5 Hz, H3, H4), 5.28 (s, 2H, $CH_2$).

$^{13}$C NMR (500 MHZ, DMSO-d6) δ 171.72 (C=O), 163.73(C2), 157.82 (C6), 136.24 (quaternary carbon of $C_6H_5$), 133.69 (C5), 128.73, 128.33, 127.97 (remaining carbons of $C_6H_5$), 110.61 (C3), 104.01 (C1), 103.03 (C4), 70.43 ($\underline{C}H_2$).

**E. 6-benzyloxy-2-hydroxy-3-nitrobenzamide**

6-benzyloxy-2-hydroxybenzamide (10 g/0.04 mol) was dissolved in 100 mL of glacial acetic acid in a 500 mL round bottom flask. This solution was cooled in an ice bath and concentrated $HNO_3$ (12 mL/0.188 mol) was added in three portions over a period of 30 minutes. The mixture was stirred for 6 hours at 0-5°C then for an additional 18 hours at room temperature.

The reaction mixture was diluted with 1.0 L of cold water and the resulting solid was recovered by filtration. The filtrate was washed with 500 mL of $H_2O$ and 3 x 50 mL portions of ethanol. The product was dried in a vacuum desiccator over $P_2O_5$. Product mass was 7.5 g.

$^1H$ NMR (500 MHZ, DMSO-$d_6$) δ 15.03 (s, 1H, 2-OH), 8.30 (d, 2H, $NH_2$), 8.10 (d, 1H, H4, J=10Hz, H4, H5) 7.50-7.35 (m, 5H, H-$C_6H_5$), 6.82(d, 1H, H5, J=10 Hz, H5, H4), 5.43 (s, 2H, $CH_2$).

$^{13}C$ NMR (500 MHZ, DMSO-$d_6$) δ 169.86 (C=0), 161.77 (C3), 158.04 (C2) 135.56 (6C), 131.38 (quaternary carbon of $C_6H_5$), 130.10 (C4), 128.74, 128.55, 127.95, 127.80 (remaining carbons of $C_6H_5$), 107.16 (C1), 103.54 (C5), 71.00 ($\underline{C}H_2$)

**F. 3-amino-6-benzyloxy-2-hydroxybenzamide**

7.3 g of 6-benzyloxy-2-hydroxy-3-nitrobenzamide was slurried in 100 mL of dry $CH_3OH$, and 1.6 g charcoal. To this was added 0.6 g $FeCl_3 \cdot 6H_2O$ and 6 mL $NH_2NH_2 \cdot H_2O$. The mixture was heated to reflux and held for 24 hours. The resulting solution was concentrated by rotary evaporation, then filtered using Celite and concentrated under vacuum at 35-40°. Product was extracted using ethyl acetate and this solution was concentrated using rotary evaporation.

$^1$H NMR (500 MHZ, DMSO-$d_6$) δ 14.32 (s, 1H, 2-OH) 8.13 (d, 2H, N$\underline{H}_2$), 7.50-7.30 (m, 5H, H-$C_6H_5$), 6.81 (d,1H, H5, J=8.5 Hz, H5, H4), 6.50 (d,1H, H4, J=8.5 Hz, H4, H5), 5.16 (s, 2H, C$\underline{H}_2$), 4.39 (s, 2H, 3-N$\underline{H}_2$).

$^{13}$C NMR (500 MHZ, DMSO-$d_6$) δ 172.41 (C=O), 151.16(C2), 148.38(C6), 136.64 (C3), 131.65 (quaternary carbon of $C_6H_5$, 128.63, 128.17, 127.98, 127.77 (remaining carbons of $C_6H_5$), 116.42 (C5), 103.06 (C1), 102.41 (C4), 70.54 (-$\underline{C}H_2$).

**G. Estradiol-17-hemisuccinate**

This compound was prepared in accordance with the procedure of Example 1.

**H. BTE2-A3**

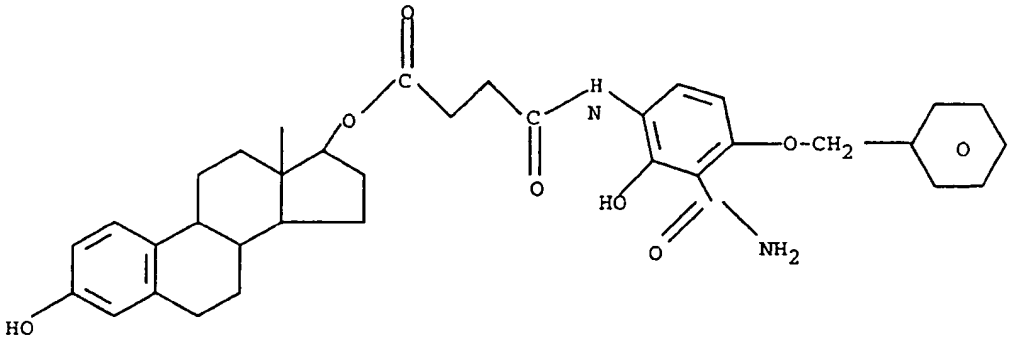
Four grams of estradiol-17-O-hemisuccinate was dissolved in 25 mL dimethylformanide (DMF) and 1.4512 g of N-hydroxybenzotriazole (HOBT) was added. The mixture was cooled to 0-5°C and was stirred for 30 minutes. Diisopropylcarbodiimide (1.354 g) was added, followed by stirring for 30 minutes at 0-5°C. A solution of 1.955 g of 3-amino-6-benzyloxy-2-hydroxybenzamide in 10 mL DMF was added and the mixture was stirred for 24 hours at room temperature. DMF was partially removed using rotary evaporation and then redissolved in ethyl acetate. This was washed with 2 x 25 mL 1N HCl, then

$H_2O$, then 2 x 25 mL portions of 10% aqueous $NaHCO_3$, then with $H_2O$ again. The ethyl acetate solution was dried over $Na_2SO_4$ and concentrated under reduced pressure. Further purification was by silica gel chromatography, developed with ethyl acetate:n-hexane, 1:1, v/v. Yield = 87%. $R_f$=0.30.

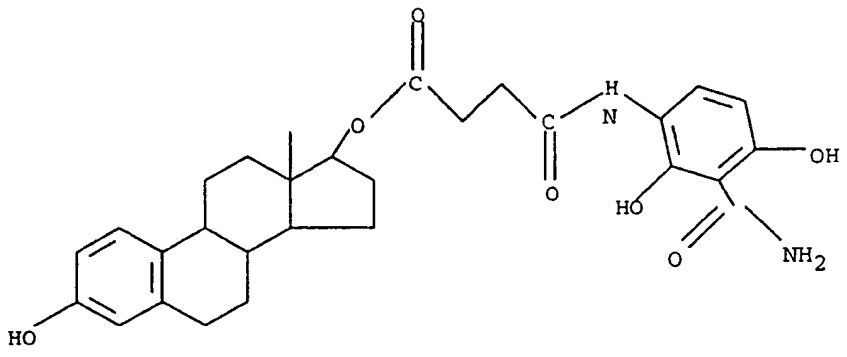
$^1H$ NMR (500 MHZ, $CDCl_3$) δ 14.46 (s, 1H, 25-O<u>H</u>), 8.40 (d, 1H, H29, J=9.00 Hz, H29, H28), 8.04 (d, 2H, 30-C-<u>N</u>$H_2$), 7.41-7.38 ($C_6H_5$), 7.09 (d, 1H, $H_2$, J=8.5 Hz, H2, H1), 6.55 (d, 1H, $H_1$ J=8.5Hz, H1, H2), 6.53 (s, 1H, H4), 6.46 (d, 1H, H28, J=9.00 Hz; H28, H29), 5.97 (s, 1H, 23-N<u>H</u>), 5.09 (s, 2H, 31-<u>C</u>$H_2$), 4.70 (t, 1H, H17), 2.77-2.70 (m, 6H, $CH_2$-6, C<u>H</u>$_2$-20, C<u>H</u>$_2$-21), 2.20-1.22 (m, 10H, remaining $CH_2$ i.e., 7,15,16,11,12), 0.77 (s, 3H, 18-<u>C</u>$H_3$).

**EXAMPLE 3**

The following scheme depicts the synthetic route for preparing the title compound as described hereinbelow:



**BTE2-A3**



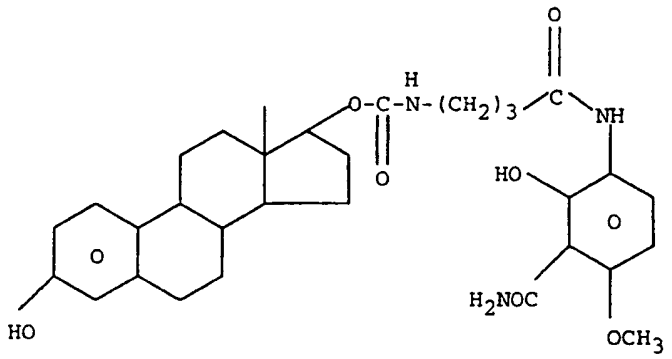
**BTE2-A1**

Synthesis of BTE2-A1

Four grams of BTE2-A3 were placed in solution with 50 mL $CH_3OH$ and 50 mL ethyl acetate. To this was added 2g Pd(C) 10% and then the slurry was placed under 40 p.s.i. of $H_2$ for 24 hours. The resultant product was filtered using Celite, then purified using silica gel chromatography, developed with ethyl acetate: n-hexane 1:1 (v/v), $R_f$ = 0.27, Yield = 2.56 g.

**EXAMPLE 4**

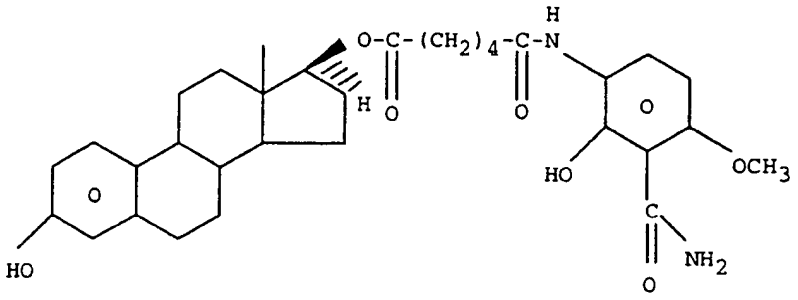
Preparation of



3-amino-2-hydroxy-6-methoxybenzamide was prepared as described hereinabove in Example 1. It is reacted with γ-butyrolactam under amide forming conditions to form the corresponding amide, i.e., 3-(4-aminobutyrylamino)-2-hydroxy-6-methoxybenzamide.

Estradiol was reacted with 3-benzyl bromide to form the 3-benzyl protected derivative, which is reacted with triphosgene to form the corresponding acid chloride. The acid chloride thus formed is reacted with the 3-(4-aminobutyrylamino)-2-hydroxy-6-methoxybenzamide produced hereinabove. Hydrogenation thereof produces the above-identified product.

**EXAMPLE 5**

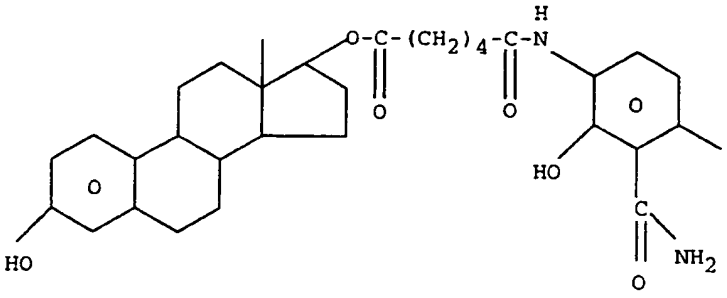


**BTE2-17B-D2**

17-β-estradiol was reacted with benzyl bromide to form the corresponding benzyl protected derivative. The product thereof was dissolved in acetone and mixed at 0-5°C with chromic oxide to form the corresponding ketone (84% yield). 1,3-propanediol and p-toluenesulfonic acid was reacted with the ketone to form the corresponding ketal (86.2% yield). The ketal was reacted with lithium aluminum hydride and aluminum chloride in THF form the corresponding 17-(3-hydroxypropoxy) derivative (73% yield). Oxidation with chromium oxide yields a 1:1 mixture of the 17-α acid and the 17β-acid. The α and β acids were separated by silica gel chromatography and the B-isomer was collected. The acid was reacted with 3-amino-2-hydroxy-6-methoxybenzamide prepared in Example 1 in the presence of diisopropylcarbodiimide followed by hydrogenation to form the above-identified product.

**EXAMPLE 6**

Preparation of

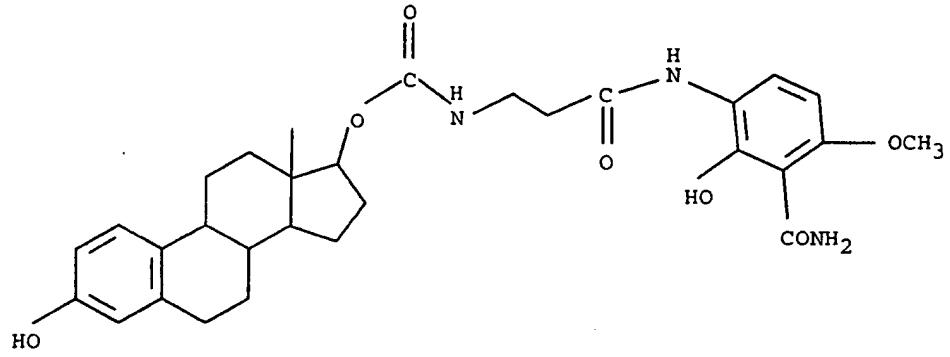



**BTE2-B2**

17β-estradiol was reacted with benzyl bromide to form the 3-benzyloxy derivative. This product is dissolved in acetone, reacted with adipic acid in the presence of p-toluene sulfonic acid under reflux to form the corresponding ester. The ester is reacted with 3-amino-2-hydroxy-6-methoxybenzamide in the presence of diisopropylcarbodiimide followed by hydrogenation to form the above-identified product.

**EXAMPLE 7**

**BTE2-E2**



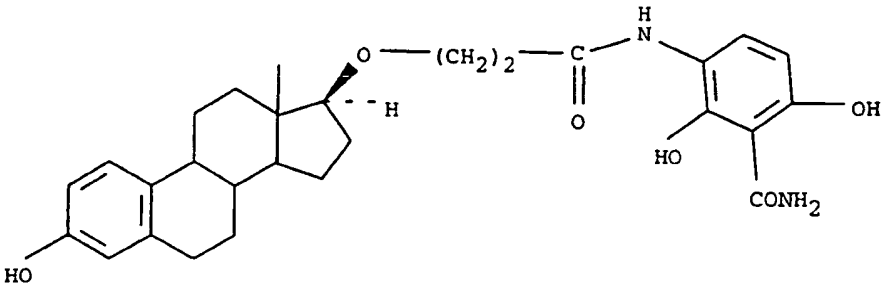

The above product was synthesized as follows:

Estradiol was reacted with benzyl bromide to form the 3-benzyl protected derivative which was reacted with triphosgene to form the corresponding acid chloride.

3-amino-2-hydroxy-6-methoxybenzamide was prepared as in Example 1. It was reacted with t-butoxycarbonyl-N-β-alanine in the presence of diisopropylcarbodimide to form the corresponding amide.

After unblocking the amino group, this product was condensed with the triphosgene activated 17-O-carbonyl chloride of 3-O-Bz-estradiol to yield BTE2-E2-3-O-Bz. Catalytic hydrogenation thereof afforded the above-identified product.

**EXAMPLE 8**




**BTE2-17B-D1 (BTE2-D1)**

The above product was prepared as follows:

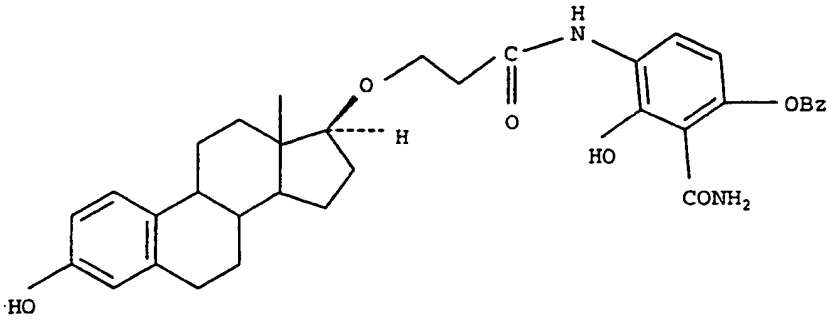
17-β-estradiol was reacted with benzyl bromide to form the corresponding benzyl protected derivative. The product thereof was dissolved in acetone and mixed at 0-5°C with chromic oxide to form the corresponding ketone (84% yield). 1,3-propanediol and p-toluenesulfonic acid was reacted with the ketone to form the corresponding ketal (86.2% yield). The ketal was reacted with lithium aluminum hydride and aluminum chloride in THF form the corresponding 17-(3-hydroxypropoxy) derivative (73% yield). Oxidation with chromium oxide yields a 1:1 mixture of the 17-α acid and the 17β-acid. The α and β acids were separated by silica gel chromatography and the B+isomer was collected.

3-amino-2-hydroxy-6-benzyl benzamide was prepared in accordance with the procedure of Example 1, A-F, except that instead of dimethylsulfoxide, as

described in Section D, benzyl bromide was used. The remainder of the synthesis described in Example 1 E and F was followed.

The 3-amino-2-hydroxy-6-benzylbenzamide was reacted with the 17β-acid in the presence of diisopropyl carbodiimide followed by hydrogenation to form the above-identified product.

**EXAMPLE 9**



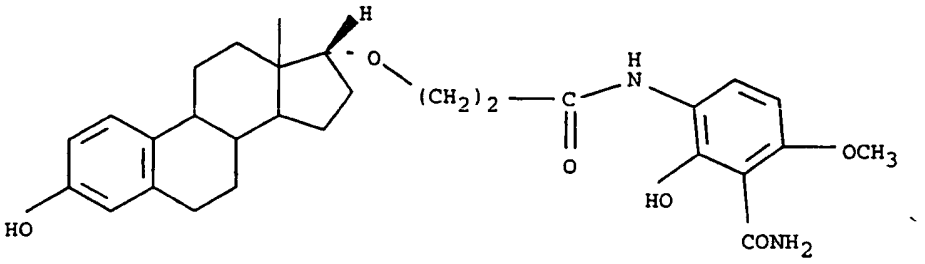

**$BTE_2$-17-β-D3 (BTE2-D3)**

The above identified product was prepared as follows:

17-β-estradiol was reacted with benzyl bromide to form the corresponding benzyl protected derivative. The product thereof was dissolved in acetone and mixed at 0-5°C with chromic oxide to form the corresponding ketone (84% yield). 1,3-propanediol and p-toluenesulfonic acid was reacted with the ketone to form the corresponding ketal (86.2% yield). The ketal was reacted with lithium aluminum hydride and aluminum chloride in THF form the corresponding 17-(3-hydroxypropoxy) derivative (73% yield). Oxidation with chromium oxide yields the corresponding acid in a mixture of 1:1 isomers of the 17α-acid and 17β-acid, which were separated using silica gel chromatography. The β acid was collected and the acid was subjected to hydrogenation to form the β-acid of estradiol-17β-O-hydroxypropylether. This product in turn was reacted

with 3-amino-2-hydroxy-6-benzyloxybenzamide prepared in Example 2 in the presence of diisopropylcarbodiimide followed by hydrogenation to form the above-identified product.

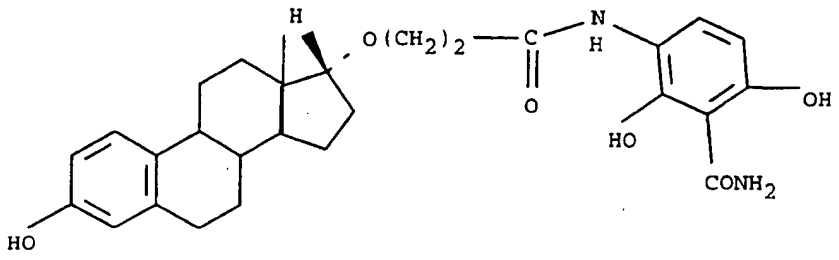
**EXAMPLE 10**




**BTE2-17-α-D2**

The above identified compound is prepared in accordance with procedure of Example 8 except 3-amino-2-hydroxy-6-methoxybenzamide and the 17-α acid were utilized.

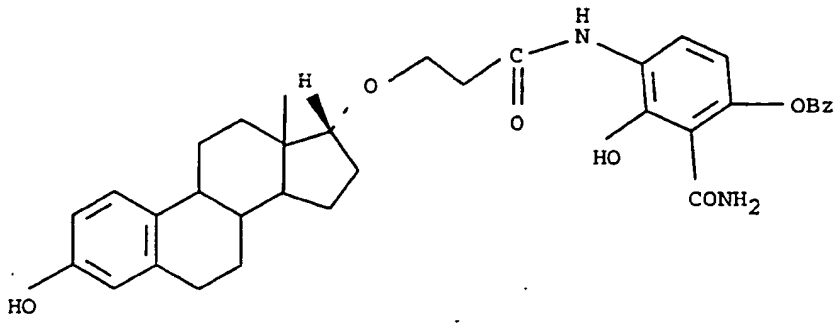
**EXAMPLE 11**




**$BTE_2$-17-α-D1**

The above-identified compound is prepared in accordance with the procedure of Example 8 except that the 17α acid is utilized.

**EXAMPLE 12**



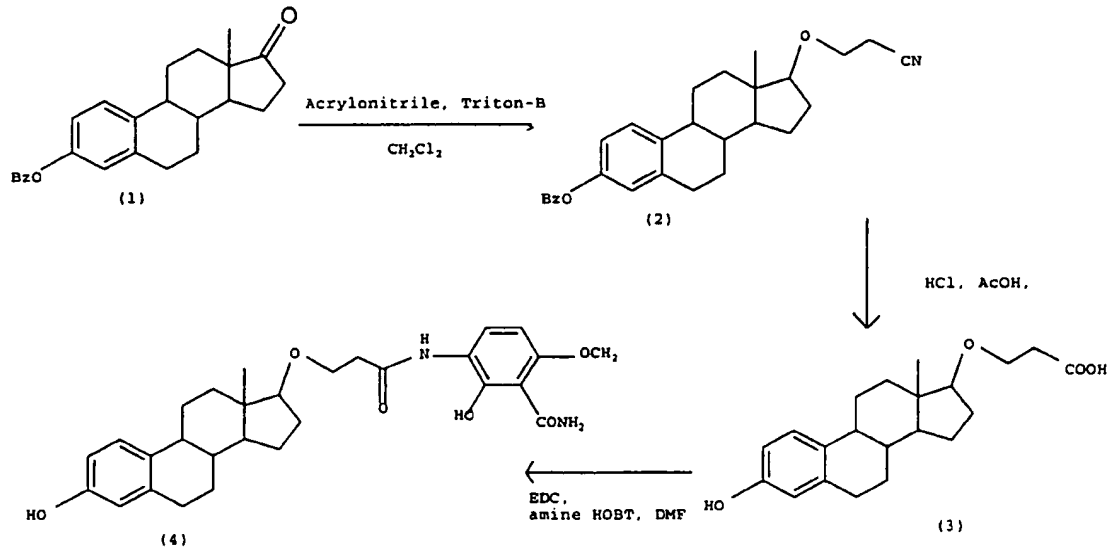

**$BTE_2$-17α-D3**

The above product is prepared in accordance with the procedure of Example 9, except that the 17-α-acid is collected and used.

**EXAMPLE 13**

**$BTE_2$-17β-D2 (BTE2-D2)**

The above-identified product was prepared by a different route than the one described in Example 5. The alternate route was as follows:

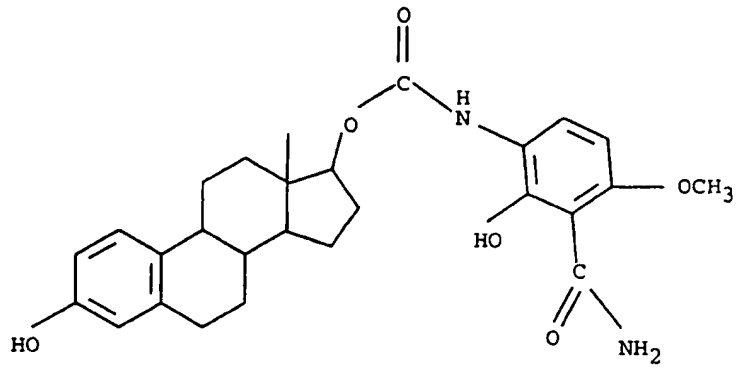



1. 2.5 g estradiol(1) was dissolved in 50 ml methylene chloride, then 6.0 ml Triton-B and 7.5 ml acrylonitrile was added. The mixture was slightly exothermic, and it was stirred at room temperature overnight. Then the solvent was evaporated and the residue was extracted with EtOAc, washed with water, dried over anhydrous sodium sulfate. The solvent was removed and the residue was purified by column chromatography using EtOAc/hexane

(1/1, v/v). Fraction with Rf=0.45 were combined and concentrated. Yield: 2.4g (80%).

2. 10 g nitrile (2), 50 ml concentrated HCl and 150 ml HOAc were refluxed 24 hours. The solvent was removed, the residue was extracted with EtOAc, washed with water, and dried over anhydrous sodium sulfate. The solvent was removed and the residue was purified by column chromatography using EtOAc/hexane (1/1, v/v). Fractions with Rf=0.45 were combined and concentrated. Yield: 3g + 1.5 g = 4.5 g.

**EXAMPLE 14**



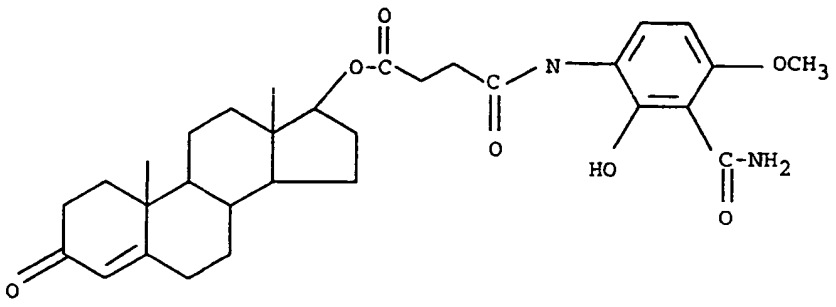

**BTE2-C2**

The above product was prepared as follows:

Estradiol was reacted with 3-benzyl bromide to form the 3-benzyl protected derivative which was reacted with trisphogene to form the corresponding acid chloride.

3-amino-2-hydroxy-6-methoxybenzamide was prepared as in Example 1 and reacted with the triphosphine activated 17-O-carbonyl chloride of 3-O-Benzylestradiol to yield BTE2-C2-O-Bz, where Bz is benzyl. Catalytic hydrogenation thereof afforded the above-identified product. MP=175°C.

**EXAMPLE 15**



The above product was prepared in accordance with the procedure of Example 1 except that 4-androstene-17-β-ol substituted for 17-β-estradiol in Part G.

**EXAMPLE 16**

**3-O-Phosphate of BTE2-D2 and BTE2-17α-D2.**

The product is prepared as follows:

The BTE2-17α-D2 product of Example 10 is reacted with O-phosphoric acid in the presence of diisopropylcarbondiimide to form the 3-O-phosphate derivative thereof.

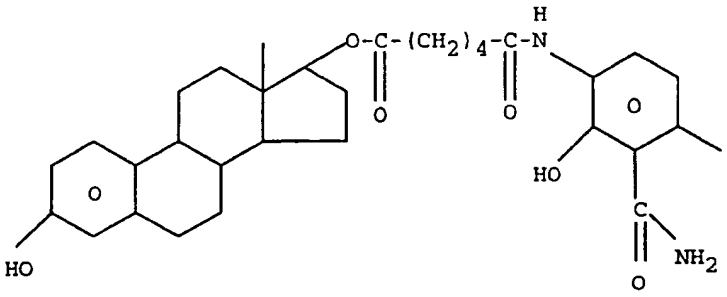
Similarly, the BTE2-17β-D2 product of Example 5 is reacted with O-phosphoric acid in the presence of diisopropylcarbodiimide to form the 3-O-phosphate derivative thereof.

**EXAMPLE 17**

**3-O-phosphate of BTE2-D3.**

The procedure of Example 16 is repeated except that the O-phosphoric acid in the presence of diisopropylcarbodiimide is reacted with the product of Example 9.

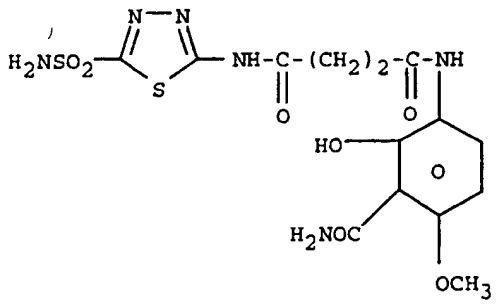
Preparation of




**BTE2-B2**

17β-estradiol was reacted with benzyl bromide to form the 3-benzyloxy derivative. This product is dissolved in acetone, reacted with adipic acid in the presence of p-toluene sulfonic acid under reflux to form the corresponding ester. The ester is reacted with 3-amino-2-hydroxy-6-methoxybenzamide in the presence of diisopropylcarbodiimide followed by hydrogenation to form the above-identified product.

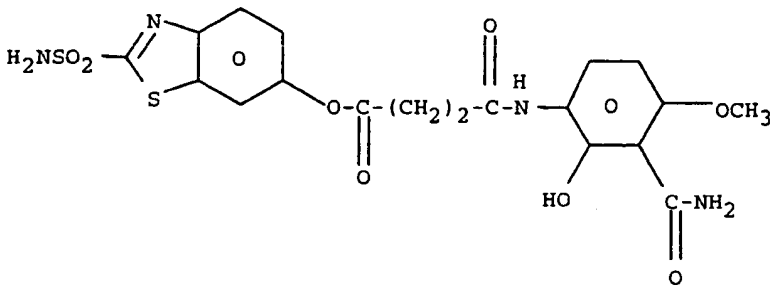
**EXAMPLE 18**



**BTCAI1-A2**

2-amino-1,3,4-thiadiazole-5-sulfonamide is reacted with succinic anhydride to form the succinamide derivative, 2-(4-carboxypropionylamino) 1,3,4-thiadiazole-5-sulfonamide. This product is reacted with 3-amino-2-hydroxy-6-methoxybenzamide in the presence of diisopropylcarbodiimide to form the above-identified compound.

**EXAMPLE 19**

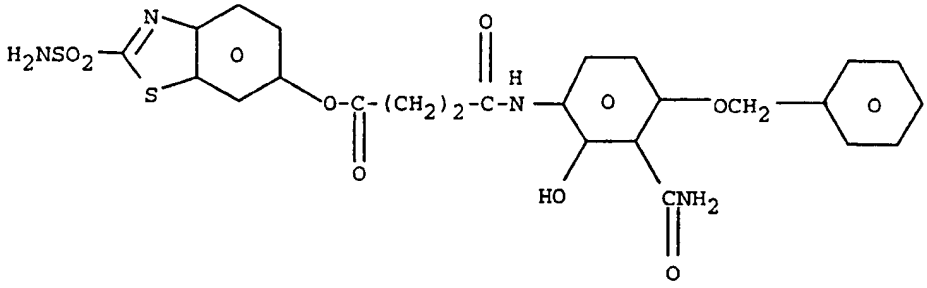


**BTCAI2-A2**

The procedure of Example 7 was followed except that 6-hydroxybenzothiazole-2-sulfonamide was used instead of 2-amino-1,3,4-thiadiazole-5-sulfonamide.

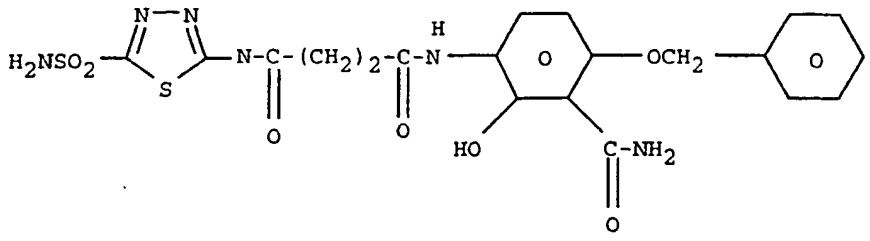
**EXAMPLE 20**

The procedure of Example 8 was followed except that 3-amino-6-benzyloxy-2-hydroxybenzamide was used instead of the 3-amino-2-hydroxy-6-methoxybenzamide to form the corresponding product having the formula:



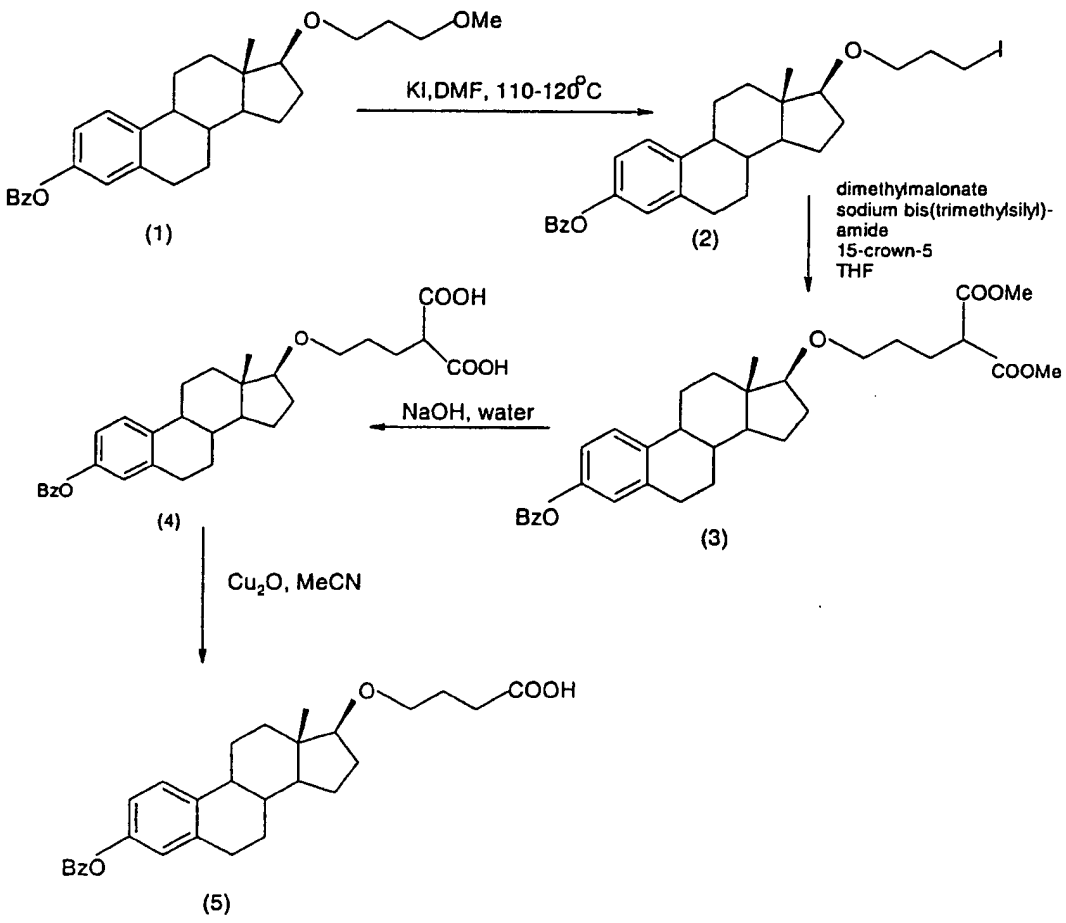
**EXAMPLE 21**

The procedure of Example 7 is repeated except that 3-amino-6-benzyloxy-2-hydroxybenzamide was used instead of 3-amino-2-hydroxy-6-methoxybenzamide to form the corresponding product



**EXAMPLE 22**

**Synthesis of the $BTE_2$-F3**



1. Synthesis of iodide (2).

1.0 g mesylate (1) was dissolved in 20 ml DMF, 1.5 g KI was added. The mixture was stirred at 110-120 °C for 6 hours. Then it was diluted with cold water and extracted with EtOAc twice. The combined organic solution was washed with brine, dried over anhydrous sodium sulfate, and evaporated to give brown oil. After silica gel column (EtOAc/hexane:1/3), 820 mg white solid (2) was collected.

2. Synthesis of diester (3).

A mixture of 302 µl dimethylmalonate, 2.64ml sodium bis(trimethylsilyl)amide (1.0M in THF) and 524µl 15-crown-5 was dissolved in 15 ml THF, and stirred at room temperature half an hour. Then a solution of 700 mg iodide (2) in 5 ml THF was added. The mixture was stirred overnight, quenched with saturated $NaHCO_3$ solution, extracted with EtOAc twice. The combined organic solution was washed with brine, dried over anhydrous sodium sulfate, and concentrated to give oil. After silica gel column (EtOAc/hexane:1/4), 600 mg oil (3) was collected.

3. Synthesis of diacid (4).

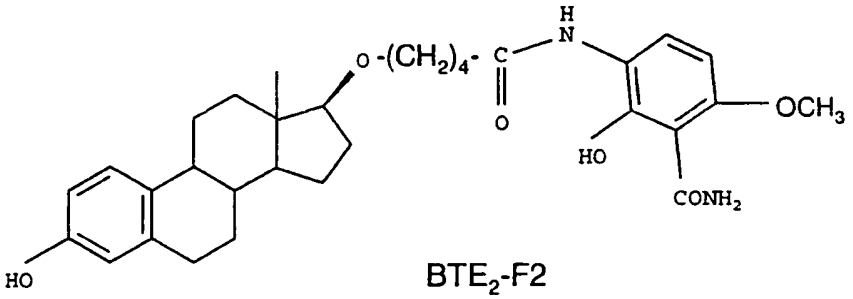
To the 600 mg diester (3) was added 30 ml 30N NaOH solution, the mixture was refluxed overnight, white precipitated appeared. After being cooled to room temperature, the mixture was treated with concentrated HCl solution and extracted with $CHCl_3$ twice. The combined organic solution was dried over anhydrous

sodium sulfate, and evaporated to give 520 mg while solid as diacid (4).

4. Synthesis of 5.

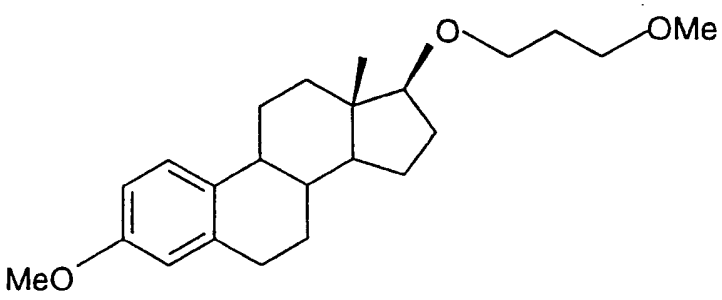
Finally, the diacid was dissolved in 50 mL acetonitrile and 100 mg of $Cu_2O$ was added. The resultant product was heated to reflux for 2 hours, then cooled and filtered. To the filtrate was added 20 mL of water, which was extracted twice with a total of 100 mL of $CHCl_3$. This solution was washed with water, dried over anhydrous sodium sulfate and evaporated to dryness, yielding 395 g of monobasic acid.

**EXAMPLE 23**



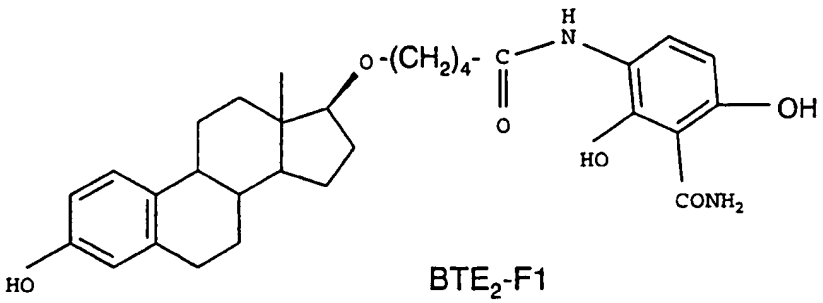

$BTE_2$-F2

The procedure of Example 22 was followed except that




was used as the starting material.

**EXAMPLE 24**




$BTE_2$-F1

The procedure of Example 22 was followed. The 6-benzyloxy derivative was converted to the corresponding 6-hydroxy derivative by catalytic hydrogenation as described in Example 3.

## EXAMPLE 25

### In vitro testing of bone targeting

The ability of these compounds to act as bone targeted pharmaceuticals was estimated initially by determination of the ability of the compounds to be bound to microcrystalline hydroxyapatite [$Ca_{10}(PO_4)_6 \cdot OH_2$] (HA) from a dilute aqueous solution.

Solutions of test compounds were constructed in 99:1, v/v, $H_2O$:dimethylsulfoxide (DMSO) at $10^{-5}$M. These solutions were taken for determination of electronic photometric absorption spectra scanning from $\lambda$=500-190nm. Absorption maxima ($\lambda_{max}$) and extinction coefficients ($\epsilon$) were determined using the Beer-Lambert law. The data is provided in Table 1 .

**TABLE 1**

| Compound | $\lambda_{max}$ (nm) | $\epsilon$ ($M^{-1} \times cm^{-1}$) |
|---|---|---|
| 2,6-dihydroxy-3-nitrobenzoic acid | 340 | 25,230 |
| 2,6-dihydroxy-3-nitrobenzamide | 395 | 9,220 |
| 17,β-Estradiol | 278 | 2,620 |
| 3-amino-2,6-dihydroxybenzamide | 338 | 2,050 |
| Tetracycline | 362 | 12,880 |

| | | |
|---|---|---|
| BTE2-A1 | 330 | 2,380 |
| BTE2-A2 | 338 | 5,100 |
| BTE2-B2 | 338 | 4,160 |

For binding determinations, 1 mL of each solution was taken and added to 0.1 mL of trishydroxymethylaminomethane (50 mM) in 1% DMSO (aq) that contained either 0 or 0.5 % (w/v) of slurried HA. These solutions and slurries were mixed for 4 minutes, then centrifuged for 3 minutes at 10,000 x g. Supernatants were taken for UV absorption spectrometry at previously determined $\lambda_{max}$, concentrations of test compound were determined and the extent of binding was calculated. Tetracycline was included as a positive control compound.

The results are given hereinbelow in Table 2.

**TABLE 2**

| Compound | % Adsorbed | Binding Index |
|---|---|---|
| 2,6-dihydroxy-3-nitrobenzoic acid | -1.4 | -3 |
| 2,6-dihydroxy-3-nitrobenzamide | -0.3 | -1 |
| 17,β-Estradiol | -4 | -8 |
| 3-amino-2,6-dihydroxy benzamide | 25.4 | 49 |
| Tetracycline | 51.6 | 100 |
| BTE2-A1 | 54.0 | 105 |

| | | |
|---|---|---|
| BTE2-A2 | 91.7 | 178 |
| BTE2-B2 | 99.1 | 192 |
| **BTE2-C2** | **100** | **>200** |
| **BTE2-D1** | **92** | **198** |
| **BTE2-D2** | **93** | **198** |
| **BTE2-D3** | **94** | **201** |
| **BTE2-E2** | **100** | **>200** |

The data in Table 2 clearly show that the compounds prepared in the present invention have a strong affinity for hydroxyapatite, stronger than even tetracycline, which is known to have a strong binding affinity to bone.

## EXAMPLE 26

**IN VIVO TESTING**

The following protocol was used for the in vivo testing:

150-170 gm female rats were utilized. Inasmuch as their ovaries produce estrogens and estrogen secretion promotes bone formation, the rats were ovariectomized (OVX) to prevent bone growth resulting therefrom. The rats were divided into different groups, which is tabulated hereinbelow:

| Group | # Animals | Surgery | Drug [Dose] |
|---|---|---|---|
| A | 8-10 | Sham | 0 |
| B | 6-8 | OVX | 0 |
| C | 6 | OVX | Positive Control (estradiol, raloxifene) [1-10,000 ug/kg] |
| D | 6 | OVX | BTEx [x ug/kg] |
| E | 6 | OVX | BTEx [10x ug/kg] |
| F | 6 | OVX | BTEx [100x ug/kg] |
| G | 6 | OVX | BTEx [1000x ug/kg] |
| H | 6 | OVX | BTEx [10,000 x ug/kg] |

In the table, the term sham is meant to connote that there was no ovariectomy in that group of rats.

The drug was administered (5°C) in corn oil and 1% DMSO to each animal 3 times a week for 6 weeks. At that time, the rats were killed by $CO_2$ asphyxiation.

The femurs were removed and weighed. The results are depicted hereinbelow.

**TABLE 3**

| | Estradiol | $BTE_2$-$A_1$ | $BTE_2$-$A_2$ | $BTE_2$-$B_2$ | $BTE_2$-$C_2$ | $BTE_2$-$D_2$ | $BTE_2$-$E_2$ |
|---|---|---|---|---|---|---|---|
| Dose µg/kg | | | | | | | |
| 0.05 | | 14±14 | | | | | |
| 0.10 | 27±2 | 24±6 | 9±6 | | 14±1 | | |
| 0.33 | | | | | | | |
| 0.5 | | | | | | | |
| 1 | 36±1 | 29±7 | -1±5 | 44±1 | 14±2 | -8±2 | 14±4 |
| 3 | | | 31±6 | | | | |
| 5 | | 64±9 | | 44±1 | | | 19±5 |
| 9 | | | 51±6 | | | | |
| 10 | 75±2 | 62±14 | 69±9 | 115±5 | 14±1 | 29±8 | 49±4 |
| 18 | 90±7 | | | | | | |
| 20 | | 69±9 | | | | | |
| 50 | | 107±5 | | | | | |
| 100 | 116±4 | 96±8 | 89±8 | | 61±2 | 22±11 | 98±8 |
| 250 | | | | | | 66±7 | 123±14 |

The weight of the femurs from the groups B-H were compared to those from Group A, that is, the

control, wherein no drug was administered and there was no removal of the ovaries from the female rat.

The values in the table hereinabove are the % increase or decrease relative to the value obtained from the control. A higher value signifies greater bone preserving activity (greater bone weight) relative to the control, while a lower weight signifies a lower bone preserving activity. A negative value indicates the femur weight from the rat treated with the drug is less than the weight of the femur from the control rat.

As clearly shown by the data, all of the compounds of the present invention that were tested exhibited strong bone preserving activity. For example with respect to BTE2-C2 in the dose range of 0.1-100 ug/kg, excellent bone preserving activity was observed. For the dose range of 0.1 - 100 ug/kg, $BTE_2$-$E_2$, $BTE_2$-$A_2$, $BTE_2$-$B_2$, $BTE_2$-$A_1$ and $BTE_2$-$D_2$ all exhibited bone preserving activity in a dose dependent fashion.

Furthermore, the compounds of the present invention exhibit affinity for bone. As shown by the results in the table, the compounds with the greatest values in the table also have the highest affinity for bone. As indicated by the data, $BTE_2$-A1, $BTE_2$-$A_2$, $BTE_2$-$B_2$, $BTE_2$-$E_2$, have high bone affinity, while $BTE_2$-$C_2$ and $BTE_2$-$D_2$ have more moderate bone affinity.

Some of the drugs, however, also exhibit another advantage, they have minimal estrogen effect. Estrogen is a female sex hormone and, among other

things, promote the development of female secondary sex characteristics, such as development and growth of the breasts. To avoid development of these "female" characteristics in the patient, it is advantageous that the drug have minimal estrogenic activity. Inasmuch as estrogen increases the size of the uterus, a comparison of uterus sizes relative to those in the sham group is indicative of the relative estrogenic activity. The results are depicted in Table 4.

**TABLE 4**

| | Estradiol | $BTE_1$-A1 | $BTE_2$-$A_2$ | $BTE_2$-$B_2$ | $BTE_2$-$C_2$ | $BTE_2$-$D_2$ | $BTE_2$-$E_2$ |
|---|---|---|---|---|---|---|---|
| Dose µg/kg | | | | | | | |
| 0.05 | | 0±1 | | | | | |
| 0.10 | 21±2 | 2±2 | -1±3 | | 4±1 | | |
| 0.33 | | | | | | | |
| 0.5 | | | | | | | |
| 1 | 51±3 | 4±2 | 7±2 | 30±4 | 5±1 | 1±1 | 1±1 |
| 3 | | | 11±3 | | | | |
| 5 | | 29±12 | | 37±5 | | | 14±1 |
| 9 | | | 19±2 | | | | |
| 10 | 71±2 | 39±5 | 47±8 | 55±6 | 5±1 | 3±1 | 31±2 |
| 18 | 64±3 | | | | | | |
| 20 | | 46±3 | | | | | |
| 50 | | 59±4 | | | | | 62±3 |
| 100 | 109±6 | | 78±11 | | 61±5 | 7±2 | 81±12 |
| 250 | | | | | | 18±2 | 103±11 |

Shading indicates uterine stimulation less than 33%.

Again, the values in Table 4 represent a % increase or decrease of uterine weight relative to the Sham group.

The data show that estradiol has a significant uterine stimulatory effect while compounds of the present invention did not stimulate the uterine as much as estradiol.

A comparison of the bone and uterine effects are depicted in Table 5:

**COMPARISON OF BONE AND UTERINE EFFECTS AT THE BONE $ED_{50}$ AND $ED_{35}$**

**TABLE 5**

| | Estradiol | $BTE_2$-$A_1$ | $BTE_2$-$A_2$ | $BTE_2$-$B_2$ | $BTE_2$-$C_2$ | $BTE_2$-$D_2$ | $BTE_2$-$E_2$ |
|---|---|---|---|---|---|---|---|
| | | | | | | | |
| **Bone Effects** | | | | | | | |
| $ED_{50}$ nmol/kg | 9 | 9 | 16 | 10 | 122 | 361 | 32 |
| $ED_{35}$ nmol/kg | 2 | 2 | 8 | 8 | 66 | 49 | 13 |
| **Uterine Effects** | | | | | | | |
| @Bone $ED_{50}$ | 58% | 24 | 30 | 35 | 50 | 10 | 40 |
| @Bone $ED_{35}$ | 46% | 0 | 0 | 30 | 30 | 2 | 20 |

Table 5 is a listing of Bone Effects of test compounds and Uterine Effects of test compounds. Bone Effects are the $ED_{50}$ and $ED_{35}$ (our chosen lowest "clinically significant" preservation of bone mass). Uterine Effects are the uterotrophic effects for test compounds at the bone $ED_{50}$ and $ED_{35}$. Thus, for example, at the $ED_{50}$ for estradiol; 58% stimulation of uterine mass was observed.

The data clearly show that $BTE_2$-$A_1$ and $BTE_2$-$D_2$ exhibit excellent separation of bone and uterine effect. For $BTE_2$-$A_1$, at $ED_{50}$ (9 nmol/kg), 24% uterine stimulation was observed. At $ED_{35}$ (2 nmol/Kg), 0% uterine stimulation was observed. For $BTE_2$-$D_2$, at $ED_{50}$ (361 nmol/Kg), 10% uterine stimulation was observed, while at $ED_{35}$ (40 nmol/kg), 2% uterine stimulation was observed. Thus, these two compounds exhibit excellent separation of bone and uterine effect.

For $BTE_2$-$A_2$, and $BTE_2$-$B_2$ there was partial separation of bone and uterine effect. With respect to $BTE_2$-$A_2$, at $ED_{50}$ (16 nmol/Kg), 30% uterine stimulation was observed, while at $ED_{35}$ (8 nmol/Kg), 2% uterine stimulation was observed. With $BTE_2$-$B_2$, at $ED_{50}$ (10 nmol/Kg), 35% uterine stimulation was observed; at $ED_{35}$ (8 nmol/Kg), 30% uterine stimulation was observed.

The preferred compounds of the present invention have maximal bone preservation effects and minimal uterine stimulatory effects as shown by $BTE_2$-$A_1$ and $BTE_2$-$D_2$. Thus, the preferred estrogens have either

an ester linkage or ether linkage between the estrogen moiety and the bridging group and an amide linkage between the bridging group and A and B. Moreover, it is preferred that the bridging group has 1-3 carbon atoms and preferably 1-2 carbon atoms separating the amide functionality at one end and the ester or ether functionality at the other end.

Although androgen compounds of the present invention are susceptible to metabolic conversion to the corresponding estrogens by aromatase, the androgenic compounds of the present invention per se exhibit minimal estrogenic effects, e.g., they exhibit minimal uterine stimulatory effects.

Compounds of the present invention have an anabolic effect. In other words, they not only help to prevent or retard bone less and they also appear to help build bone. Compounds of the present invention in which this trait is especially noted are those in which YEV contains an ether linkage at the Y end. This is shown by the following experiment.

**EXAMPLE 26**

The procedure of Example 25 was followed using BTE2-D3 as the drug. More specifically, 150-170gm rats were utilized which were ovariectomized (OVX) to prevent bone growth resulting therefrom. The rats were divided into different groups tabulated hereinbelow:

| Group | # Animals | Surgery | Drug [Dose] |
|---|---|---|---|
| A | 8-10 | Sham | 0 |
| B | 6-8 | OVX | 0 |
| C | 6 | OVX | Positive Control (estradiol, raloxifene) [1-10,000 ug/kg] |
| D | 6 | OVX | BTEx [x ug/kg] |
| E | 6 | OVX | BTEx [10x ug/kg] |
| F | 6 | OVX | BTEx [100x ug/kg] |
| G | 6 | OVX | BTEx [1000x ug/kg] |
| H | 6 | OVX | BTEx [10,000 x ug/kg] |

In the table, the term sham is meant to connote that there was no ovariectomy in that group of rats.

The drug was administered (5°C) in corn oil and 1% DMSO to each animal 3 times a week for 6 weeks. At that time, the rats were killed by $CO_2$ asphyxiation. The femurs and uteri were removed and weighed. The results are depicted hereinbelow.

**Effects of $BTE_2$-D2 given p.o.**

| Treatment | Uterine Mass g/kg BW | BMD-pQCT | Femur Mass g/kg BW | Density $g/cm^3$ |
|---|---|---|---|---|
| Control | 1.80 ± 0.65 | 479 ± 18 | 2.93 ± 0.11 | 1.49± 0.02 |
| OVX | 0.28 ± 0.07 | 258 ± 18 | 2.62 ± 0.12 | 1.47 ± 0.02 |
| Raloxifene 1 mg/kg | 0.31 ± 0.06 | 292 ± 17 | 2.81 ± 0.07 | 1.48 ± 0.02 |
| Raloxifene 10 mg/kg | 0.31 ± 0.05 | 335 ± 23 | 2.87 ± 0.10 | 1.48 ± 0.01 |
| $BTE_2$-D2 30 µg/kg p.o. | 0.73 ± 0.03 | 343 ± 20 | 2.85 ± 0.04 | 1.47 ± 0.01 |
| $BTE_2$-D2 100 µg/kg p.o. | 1.02 ± 0.06 | 407 ± 25 | 3.02 ± 0.07 | 1.48 ± 0.02 |
| $BTE_2$-D2 300 µg/kg p.o. | 1.30 ± 0.15 | 452 ± 46 | 3.07 ± 0.12 | 1.48 ± 0.03 |
| $BTE_2$-D2 1000 µg/kg p.o. | 1.35 ± 0.25 | 601 ± 61 | 3.20 ± 0.12 | 1.51 ± 0.02 |
| $BTE_2$-D2 3000 µg/kg p.o. | 1.61 ± 0.31 | 620 ± 53 | 3.31 ± 0.07 | 1.52 ± 0.02 |
| $BTE_2$-D2 10000 µg/kg p.o. | 1.58 ± 0.25 | 611 ± 22 | 3.31 ± 0.10 | 1.53 ± 0.25 |

A comparison of the distal Femoral Density, as determined by Quantitative Computer Tomography ("QCT"), of BTE2-D3, BTE2-D2 and BTE2-D1, and two controls were graphically depicted as a function of dose. The results

are indicated in Figure 1. As clearly shown, the bone density of the distal femoral increased as the dose of BTE2-D3 and BTE2-D2 and BTE2-D1 was increased, with those rats treated with BTE2-D2 and BTE2-D3 exhibiting higher distal femoral density than the control. Moreover, the bone density of the distal femoral in rats treated with BTE2-D3 was extraordinarily high.

Inasmuch as bone density is not necessarily the equivalent to bone strength, an additional test, the blunt indentation force of the distal femoral metaphysis, was also performed.

The results are graphically depicted in Figure 2.

Raloxifene, at a dose of 1 mg/mkg, had a small (30%) effect. Extradiol was able to completely protect against osteopenia. However, these data clearly show that BTE2-D3 had a substantially greater effect on bone density than did the parent estradiol. The bone strength data show a greater than 100% protection, indicating these changes in bone density are accompanied by changes in bone-mechanical competence.

**EXAMPLE 27**

The procedure of Example 26 was repeated except BTE2-D2 was given s.c. to the rats. The results are indicated hereinbelow.

**Effects of $BTE_2$-D2 given s.c.**

| Treatment | Uterine Mass g/kg BW | BMD - pQCT | Femur Mass g/kg BW | Density $g/cm^3$ |
|---|---|---|---|---|
| Control | 1.39 ± 0.55 | 425 ± 11 | 2.92 ± 0.11 | 1.49 ± 0.01 |
| OVX | 0.18± 0.02 | 222 ± 11 | 2.56 ± 0.14 | 1.46 ± 0.02 |
| Estradiol 10 ug/kg | 0.78 ± 0.16 | 297 ± 15 | 2.91 ± 0.02 | 1.48 ± 0.01 |
| Estradiol 100 ug/kg | 1.03 ± 0.11 | 399 ± 24 | 2.97 ± 0.09 | 1.49 ± 0.01 |
| $BTE_2$-D2 10 ug/kg s.c. | 0.69 ± 0.18 | 285± 20 | 2.79 ± 0.14 | 1.49 ± 0.01 |
| $BTE_2$-D2 100 ug/kg s.c. | 1.15 ± 0.36 | 422 ± 25 | 2.97 ± 0.16 | 1.46 ± 0.02 |
| $BTE_2$-D2 1000 ug/kg s.c. | 1.49 ± 0.44 | 552 ± 95 | 3.21 ± 0.08 | 1.53 ± 0.02 |
| $BTE_2$-D2 10000 ug/kg s.c. | 2.09 ± 0.47 | 484 ± 21 | 3.49 ± 0.11 | 1.51 ± 0.03 |

As the data shows the BTE2-D2 is orally active and completely antagonizes the bone loss that occurs after ovariectomization. The animals treated with BTE2-D2 show much greater bone stimulation than do the control animals.

In addition, there is significant uterine stimulation.

The above preferred embodiments and examples are given to illustrate the scope and spirit of the present invention. These embodiments and examples will make apparent to those skilled in the art other embodiments and examples. These other embodiments and examples are within the contemplation of the present invention.

Therefore, the present invention should be limited only by the appended claims.

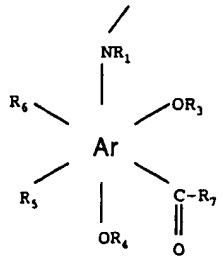
**WHAT IS CLAIMED IS**:

1. A compound of the formula:

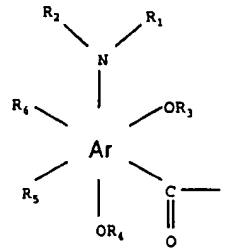
A-C(=O)-X-Y-E-V-Q or B-$NR_8$-X-Y-E-V-Q

or pharmaceutically acceptable salts thereof wherein

A is




B is




;

Ar is aryl group containing 6-10 ring carbon atoms or the corresponding saturated or partially saturated cyclic group;

$R_1$ is hydrogen, lower alkyl or aryl lower alkyl;

$R_2$ is hydrogen, lower alkyl or aryl lower alkyl;

$R_3$ is hydrogen or lower alkyl;

$R_4$ is hydrogen, aryl, aryl lower alkyl, or lower alkyl;

$R_5$ and $R_6$ are independently hydrogen or lower alkyl or $R_5$ and $R_6$ taken together with the carbon atoms to which they are bonded form a ring containing 4-10 ring carbon atoms and up to a total of 18 carbon atoms;

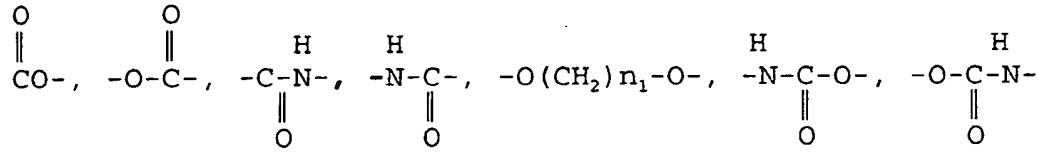
$R_7$ is hydroxy, lower alkoxy, or $NR_8R_9$;

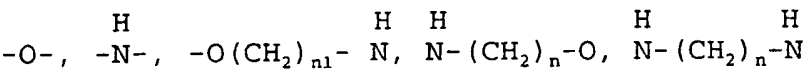
$R_8$ and $R_9$ are independently hydrogen or lower alkyl;

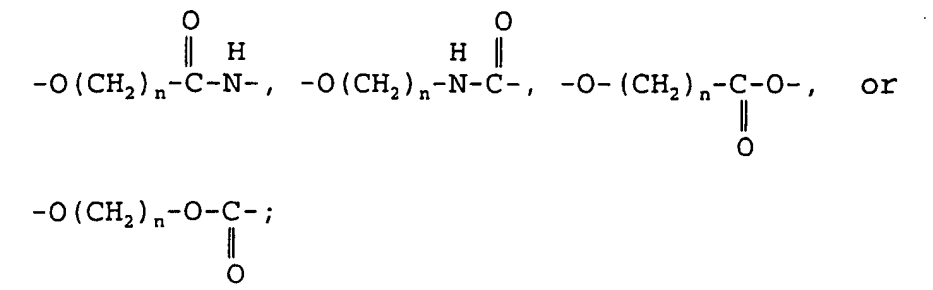
X is an alkylene group containing from 1-10 carbon atoms on the main chain and up to a total of 20 carbon atoms;

V is  ;

Y-E-V is





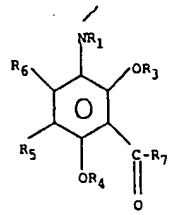


Q is a bone active domain less a VH group, said bone active domain being selected from the group consisting of carbonic anhydrase inhibitors, estrogens, androgens, D vitamins, HMG-CoA reductase inhibitors, DHEA, proton pump inhibitors, PTH, $T_3$, $T_4$, prostaglandins, and pharmaceutically acceptable salts thereof and mixtures thereof and said bone active domain containing a VH functional group thereon or a functional group capable of being converted to VH, whereby Q is bonded to XYE through the V group;

n is 0-6; and

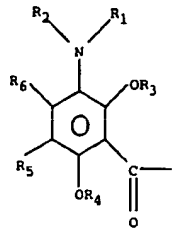
$n_1$ is 1-6

2. The compound according to Claim 1 wherein A is



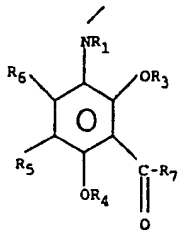
or the corresponding cyclohexyl, cyclohexenyl or cyclohexadienyl, and

B is

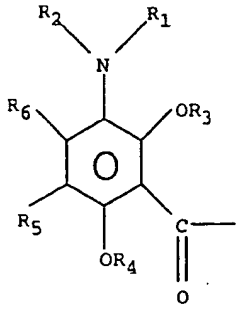


or the corresponding cyclohexyl, cyclohexenyl, or cyclohexadienyl.

3. The compound according to Claim 1 wherein A is



and B is



4. The compound according to Claim 1 wherein the bone active domain is vitamin D, a sex hormone, a steroidal compound exhibiting an androgen or estrogen effect when administered to animal, a carbonic anhydrase inhibitor, a proton pump inhibitor or a HMG CoA reductase inhibitor.

5. The compound according to Claim 1 wherein $R_7$ is $NR_8R_9$.

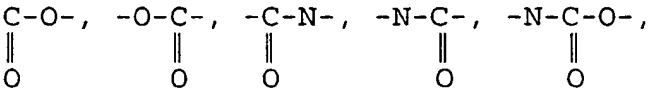
6. The compound according to Claim 1 wherein $R_3$ is hydrogen.

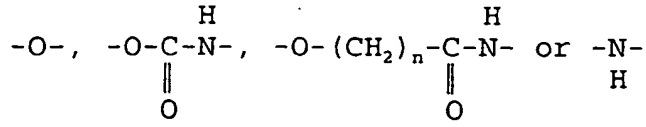
7. The compound according to Claim 1 wherein $R_5$ and $R_6$ are independently hydrogen or lower alkyl.

8. The compound according to Claim 1 wherein X is an alkylene bridge containing 2-6 carbon atoms.

9. The compound according to Claim 8 wherein X contains 2-4 carbon atoms.

10. The compound according to Claim 1

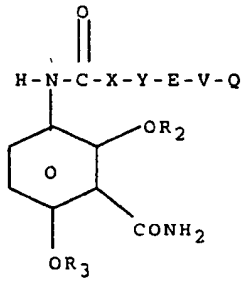
wherein Y-E-V is 



11. The compound according to claim 1 wherein the carbonic anhydrase inhibitor is 2-amino-1-3,4-thiadiazole-5-sulfonamide or 5-hydroxybenzothiazole sulfonamide; the androgenic agent is testosterone or androstenedione; and the estrogenic agent is estriol or estradiol.

12. The compound according to Claim 1 wherein the steroid exhibiting an androgen or estrogen effect when administered to animals is DHEA.

13. The compound according to Claim 1 of the formula



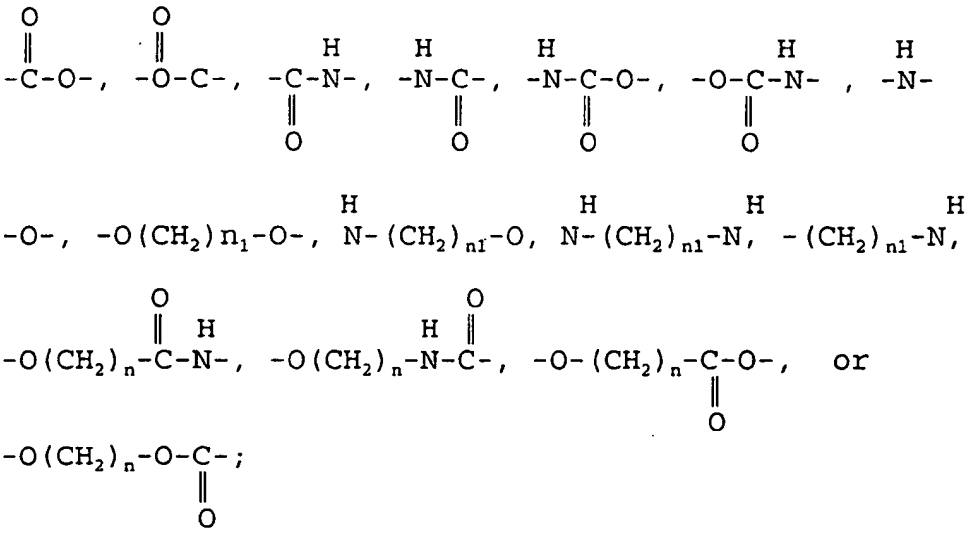
wherein

$R_3$ is hydrogen or lower alkyl;

$R_4$ is hydrogen, lower alkyl, aryl or lower arylalkyl;

X is an alkylene group containing 1-6 carbon atoms;

Y-E-V is



Q is a bone active domain less a VH group, said bone active domain being selected from the group consisting of carbonic anhydrase inhibitors, estrogens, androgens, D vitamins, HMG-CoA reductase inhibitors, DHEA proton pump inhibitors, parathyroid hormone, $T_3$, $T_4$, prostaglandins, and pharmaceutically acceptable salts thereof and mixtures thereof, and said bone active domain containing a VH functional group thereon or a functional group

capable of being converted to VH, whereby Q is bonded to XYE through the V group;

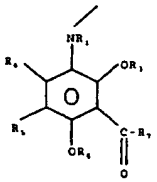
n is 0-6; and

$n_1$ is 1-6.

14. The compound according to Claim 13 wherein the estrogenic agent is estriol or estradiol; the androgenic agent is testosterone or androstenedione; and the carbonic anhydrase inhibitor is 2-amino-1,3,4-thiadiazole-5-sulfonamide or 5-hydroxybenzothiazole sulfonamide.

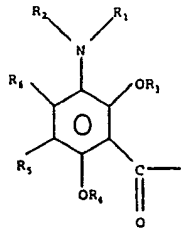
15. The compound according to Claim 1 wherein V is O.

16. The compound according to Claim 13 wherein A is



or the corresponding cyclohexyl, cyclohexenyl or cyclohexadienyl; and

B is



or the corresponding cyclohexyl, cyclohexenyl or cyclohexadienyl.

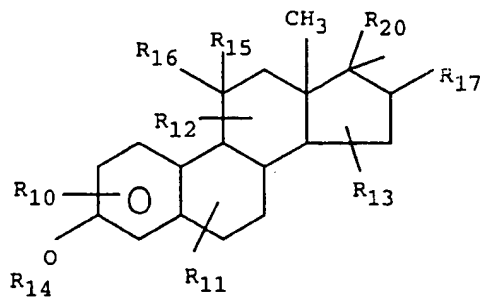
17. The compound according to Claim 15 wherein $R_7$ is $NR_8R_9$.

18. The compound according to Claim 15 wherein $R_3$ is hydrogen.

19. The compound according to Claim 15 wherein $R_5$ and $R_6$ are independently hydrogen or lower alkyl.

20. The compound according to Claim 15 wherein X is an alkylene group containing 1-6 carbon atoms.

21. The compound according to Claim 15 wherein Q is




or pharmaceutically acceptable salts thereof,

wherein

$R_{10}$, $R_{11}$, $R_{12}$ and $R_{13}$ are independently hydrogen, hydroxy, lower alkyl, lower alkoxy, amino, diloweralkylamino, lower alkylamino, or halo; and

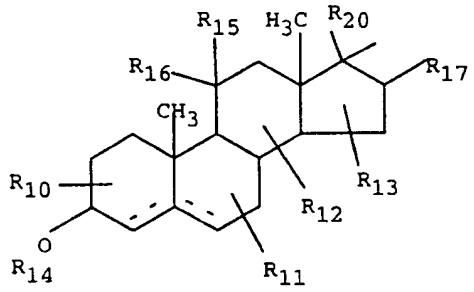
$R_{14}$ is hydrogen, $PO_3H_2$, lower alkyl, aryl, or lower aryl alkyl;

$R_{20}$ is hydrogen, loweralkyl, or lower alkynyl;

$R_{15}$ and $R_{16}$ are independently hydrogen, lower alkyl, halo, hydroxy, lower alkoxy, amino, lower alkyl amino, or diloweralkylamino or $R_{15}$ and $R_{16}$ taken together form an oxo; and

$R_{17}$ is hydroxy, oxo, hydrogen, halo, hydroxy, lower alkoxy, amino, lower alkylamino, diloweralkylamino, lower alkynyl or lower alkenyl.

22. The compound according to Claim 1 wherein Q is



or pharmaceutically acceptable salts thereof,

wherein

$R_{10}$, $R_{11}$, $R_{12}$ and $R_{13}$ are independently hydrogen, hydroxy, lower alkyl, lower alkoxy, amino, diloweralkylamino, lower alkylamino, or halo; and

$R_{14}$ is hydrogen, lower alkyl, aryl or lower aryl alkyl or $HOPO_2$, or $OR_{14}$ is oxo and --- means a carbon-carbon double bond may be present or absent, provided that the compound contains at most only 1 carbon-carbon double bond;

$R_{20}$ is hydrogen, loweralkyl, or lower alkynyl;

$R_{15}$ and $R_{16}$ are independently hydrogen, lower alkyl, halo, hydroxy, lower alkoxy, amino, lower alkyl amino, or diloweralkylamino or $R_{15}$ and $R_{16}$ taken together form an oxo; and

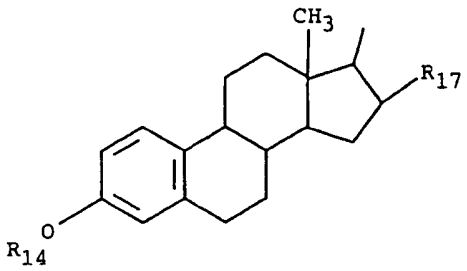
$R_{17}$ is hydroxy, oxo, hydrogen, halo, hydroxy, lower alkoxy, amino, lower alkylamino, diloweralkylamino, lower alkynyl or lower alkenyl.

23. The compound according to Claim 21 or 22 wherein $R_{10}$, $R_{11}$, $R_{12}$ and $R_{13}$ and $R_{20}$ are hydrogen.

24. The compound according to Claim 21 or 22 wherein X is an alkylene chain containing 2-6 carbon atoms.

25. The compound according to Claim 23 wherein X is an alkylene chain containing 1-3 carbon atoms.

26. The compound according to Claim 21 wherein Q is

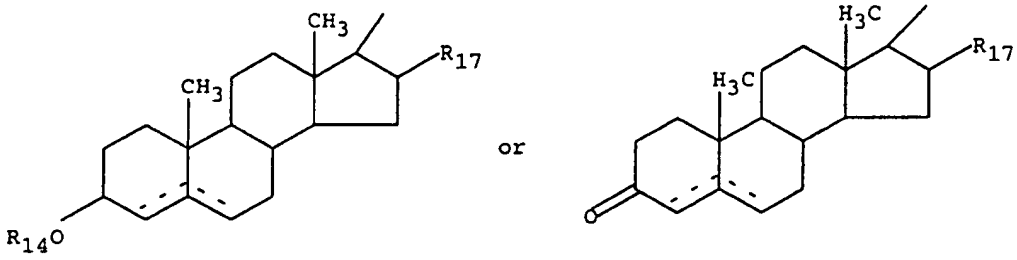



or pharmaceutically acceptable salts thereof,

wherein

$R_{17}$ is hydrogen or hydroxy and $R_{14}$ is lower alkyl, hydrogen, aryl or aryl lower alkyl or $PO_3H$.

27. The compound according to Claim 22 wherein Q is

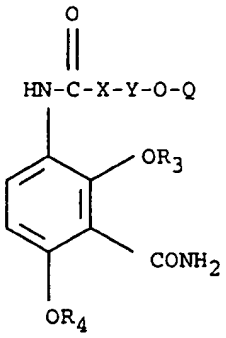


or pharmaceutically acceptable salts thereof,

wherein

$R_{17}$ is hydrogen or hydroxy and $R_{14}$ is lower alkyl, hydrogen, aryl or aryl lower alkyl or $PO_3H_2$.

28. The compound according to Claim 15 having the formula:



or pharmaceutically acceptable salts thereof.

wherein

$R_4$ is hydrogen or lower alkyl, aryl or aryl lower alkyl;

$R_3$ is hydrogen or lower alkyl;

X is an alkylene group containing 1-6 carbon atoms;

Y is C or a chemical bond; and
‖
O

Q is a bone active domain less a VH group, said bone active domain being selected from the group consisting of carbonic anhydrase inhibitors, estrogens, androgens, D vitamins, HMG-CoA reductase inhibitors, DHEA proton pump inhibitors, PTH, $T_3$, $T_4$, prostaglandins, and pharmaceutically acceptable salts thereof and mixtures thereof and said bone active domain containing a OH functional group thereon or a functional group capable of being converted to OH.

29. The compound according to Claim 28 wherein the sex hormone is estrogen.

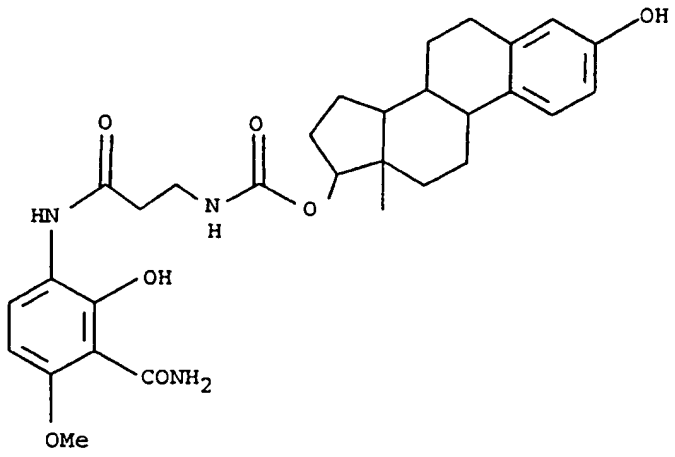
30. The compound according to Claim 29 wherein the estrogen is estradiol or estriol.

31. The compound according to Claim 28 wherein the sex hormone is androgen.

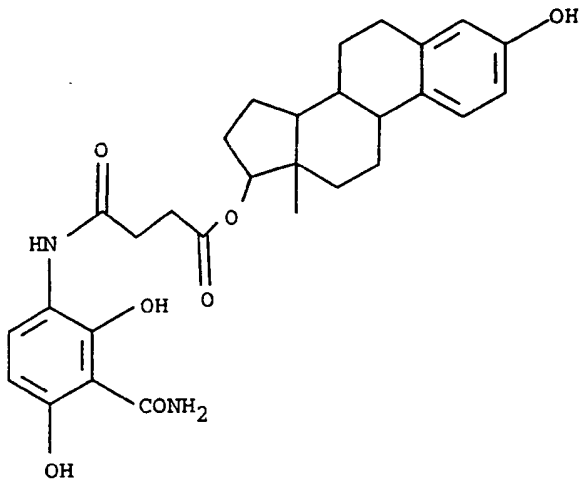
32. The compound according to Claim 31 wherein the androgen is testosterone, androstenedione or DHEA.

33. The compound according to Claim 25 wherein X is a chemical bond or an alkylene having 1 to 3 carbon atoms.

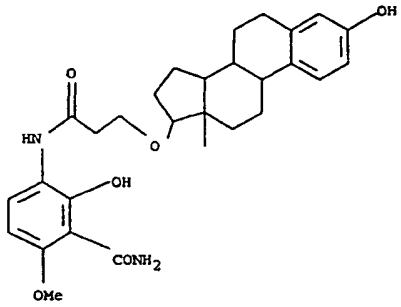
34. The compound according to Claim 1 which is:



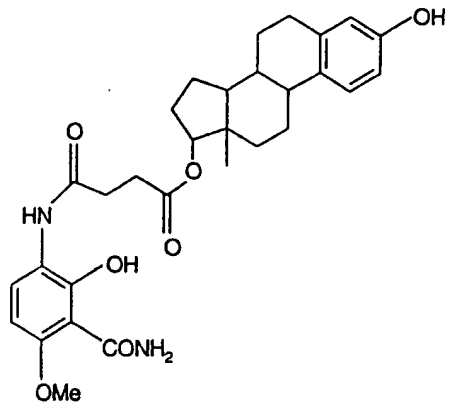

35. The compound according to Claim 1 which is:



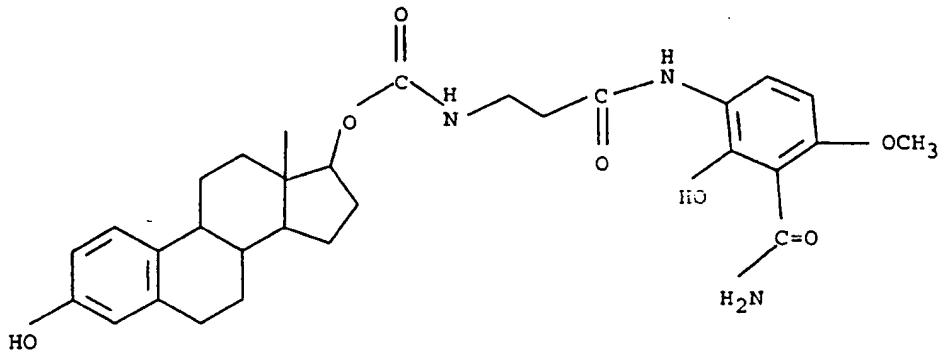

36. The compound according to Claim 1 which is:



37. The compound according to Claim 1 which is:




38. The compound according to Claim 1 which is:



39. The compound according to Claim 1 which is BTE2-B2.

40. The compound according to Claim 1 which is BTE2-C2.

41. The compound according to Claim 1 which additionally exhibits minimal estrogen effect.

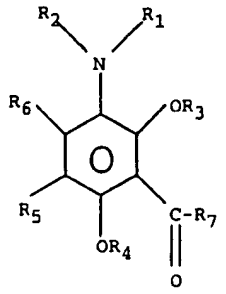
42. A pharmaceutical composition comprising a pharmaceutically effective amount of a compound according to Claim 1 and a pharmaceutically acceptable carrier therefor.

43. A method for the treatment or prophylaxis of degenerative bone disorders in an animal in need of such treatment which comprises administering thereto an effective amount of a compound according to Claim 1.

44. The method according to Claim 43 wherein said animal is a mammal.

45. The method according to Claim 43 wherein the degenerative bone disease is osteoporosis.

46. A compound of the formula



or the corresponding cyclohexyl, cyclohexenyl or cyclohexadienyl;

wherein

$R_1$ and $R_2$ are independently hydrogen, lower alkyl or aryl lower alkyl,

$R_3$ is hydrogen or lower alkyl,

$R_4$ is hydrogen, lower alkyl, aryl lower alkyl or aryl,

$R_5$ and $R_6$ are independently hydrogen or lower alkyl, or $R_5$ and $R_6$ taken together with the carbon atoms to which they are attached form a ring containing up to 10 ring carbon atoms and up to a total of 18 carbon atoms,

$R_7$ is hydroxy, lower alkoxy or $NR_8R_9$ and

$R_8$ and $R_9$ are independently hydrogen or lower alkyl.

47. The compound according to Claim 46 wherein $R_5$ and $R_6$ are both hydrogen.

48. The compound according to Claim 46 wherein $R_7$ is $NR_8R_9$.

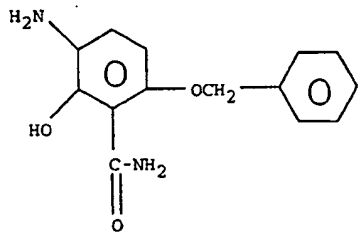
49. The compound according to Claim 48 wherein $R_8$ and $R_9$ are both hydrogen.

50. The compound according to Claim 46 wherein $R_3$ is hydrogen.

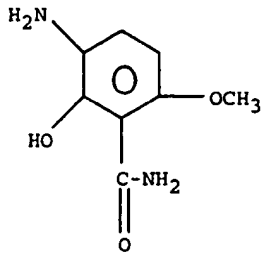
51. The compound according to Claim 46 wherein $R_5$ and $R_6$ are hydrogen, $R_7$ is amino, $R_3$ is hydrogen, $R_1$ and $R_2$ are both hydrogen and $R_4$ is lower alkyl or hydrogen.

52. The compound according to Claim 51 wherein $R_4$ is hydrogen.

53. The compound according to Claim 46 which is

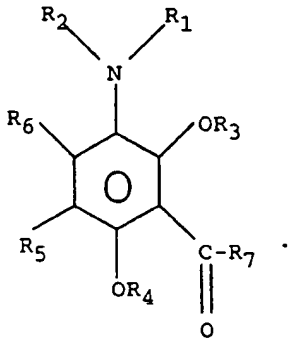


54. The compound according to Claim 46 which is



55. The compound according to Claim 1 which is BTE2-A2, BTE2-A3, BET2-B2, BTE2-A1, BTCAI-1-A2 or BTCAI2-A2.

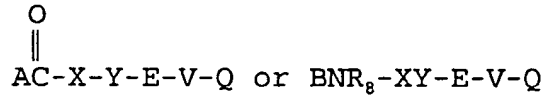
56. The compound according to Claim 46 wherein the compound is



57. The compound according to Claim 1 wherein the sex hormones is a steroidal androgen or steroidal estrogen.

58. The compound according to Claim 1 wherein the sex hormone and Vitamin D are naturally occurring.

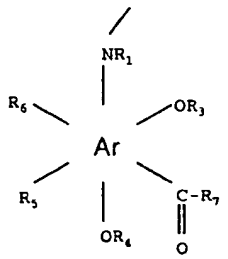
59. A method of promoting the growth of bones in a patient which comprises administering to said patient a compound of the formula:



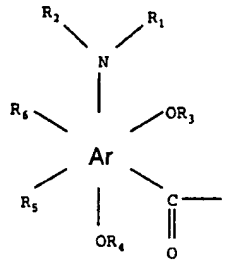
or pharmaceutically acceptable salts

wherein

A is



B is




;

Ar is aryl group containing 6-10 ring carbon atoms or the corresponding saturated or partially saturated cyclic group;

$R_1$ is hydrogen, lower alkyl or aryl lower alkyl;

$R_2$ is hydrogen, lower alkyl or aryl lower alkyl;

$R_3$ is hydrogen or lower alkyl;

$R_4$ is hydrogen, aryl, aryl lower alkyl, or lower alkyl;

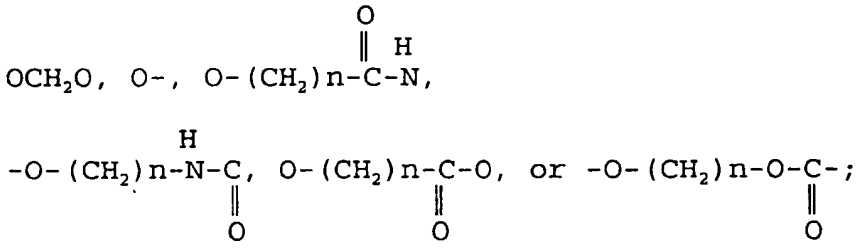
$R_5$ and $R_6$ are independently hydrogen or lower alkyl or $R_5$ and $R_6$ taken together with the carbon atoms to which they are bonded form a ring containing 4-10 ring carbon atoms and up to a total of 18 carbon atoms;

$R_7$ is hydroxy, lower alkoxy, or $NR_8R_9$;

$R_8$ and $R_9$ are independently hydrogen or lower alkyl;

X is an alkylene group containing from 1-10 carbon atoms on the main chain and up to a total of 20 carbon atoms;

Y-E-V is

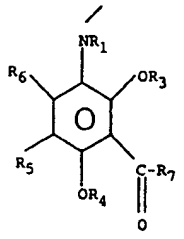


Q is a bone active domain less a VH group, said bone active domain being selected from the group consisting of carbonic anhydrase inhibitors, estrogens, androgens, D vitamins, HMG-CoA reductase inhibitors, DHEA proton pump inhibitors, PTH, $T_3$, $T_4$, prostaglandins, and pharmaceutically acceptable salts thereof and mixtures thereof, and said bone active domain containing a VH functional group thereon or a functional group capable of being converted to VH, whereby Q is bonded to XYE through the V group; and

n is 0-6.

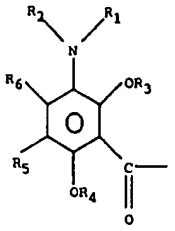
60. The method of Claim 59 wherein

A is



or the corresponding cyclohexyl, cyclohexenyl or cyclohexadienyl, and

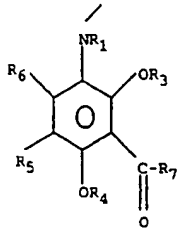
B is



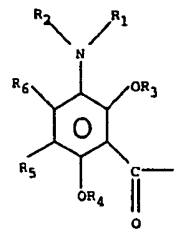
.

61. The method of Claim 60 wherein

A is



and B is



62. The method according to Claim 59 wherein the bone active domain is a vitamin D, a sex hormone, a steroidal compound exhibiting an androgen or estrogen effect when administered to animal, a carbonic anhydrase inhibitor, a proton pump inhibitor or a HMG CoA reductase inhibitor.

63. The method according to Claim 59 wherein

YEV is $O-(CH_2)n-NH-\overset{\overset{O}{\|}}{C}$, $O-(CH_2)n-\overset{\overset{O}{\|}}{C}-NH-$, $O-(CH_2)n-\overset{\overset{O}{\|}}{C}-O$, or

$-O(CH_2)n-O-\overset{\overset{O}{\|}}{C}-$.

64. The method according to Claim 59 wherein the bone active domain is an estrogen.

65. The method according to Claim 1 wherein the compound is BTE2-D1, BTE2-D2, BTE2-D3, BET2-F1, BTE2-F2 or BTE2-F3.

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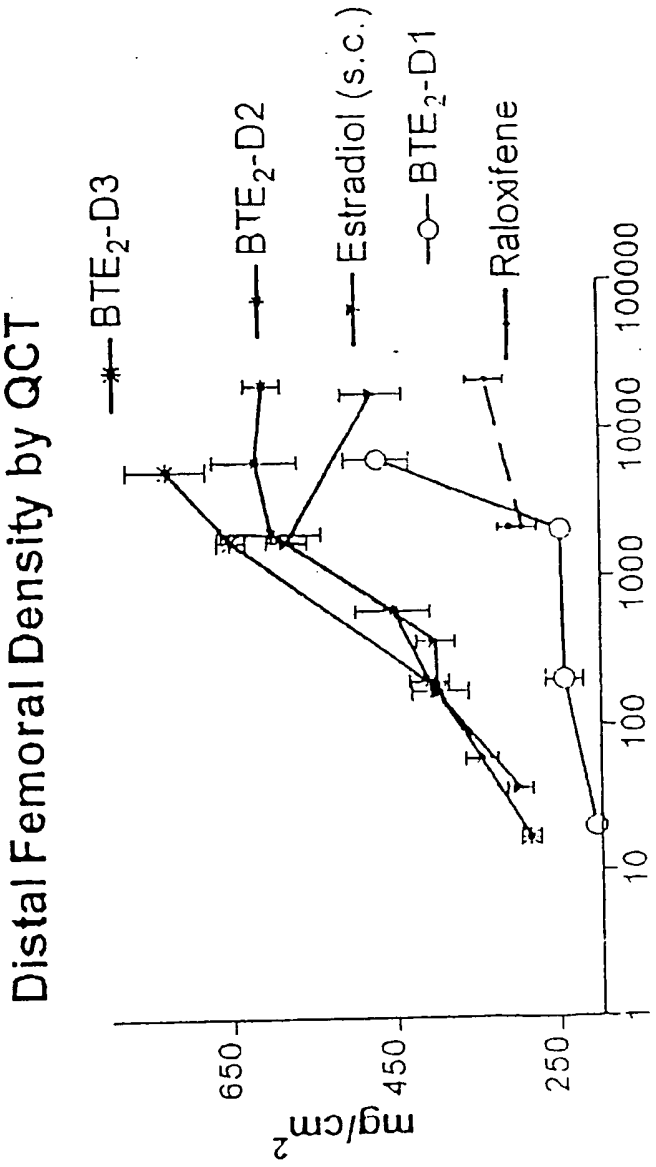
Distal Femoral Density by QCT
BTE2-D3
BTE2-D2
Estradiol (s.c.)
BTE2-D1
Raloxifene
mg/cm2
650
450
250
1
10
100
1000
10000
100000
Dose [nmol/kg]

FIGURE 1

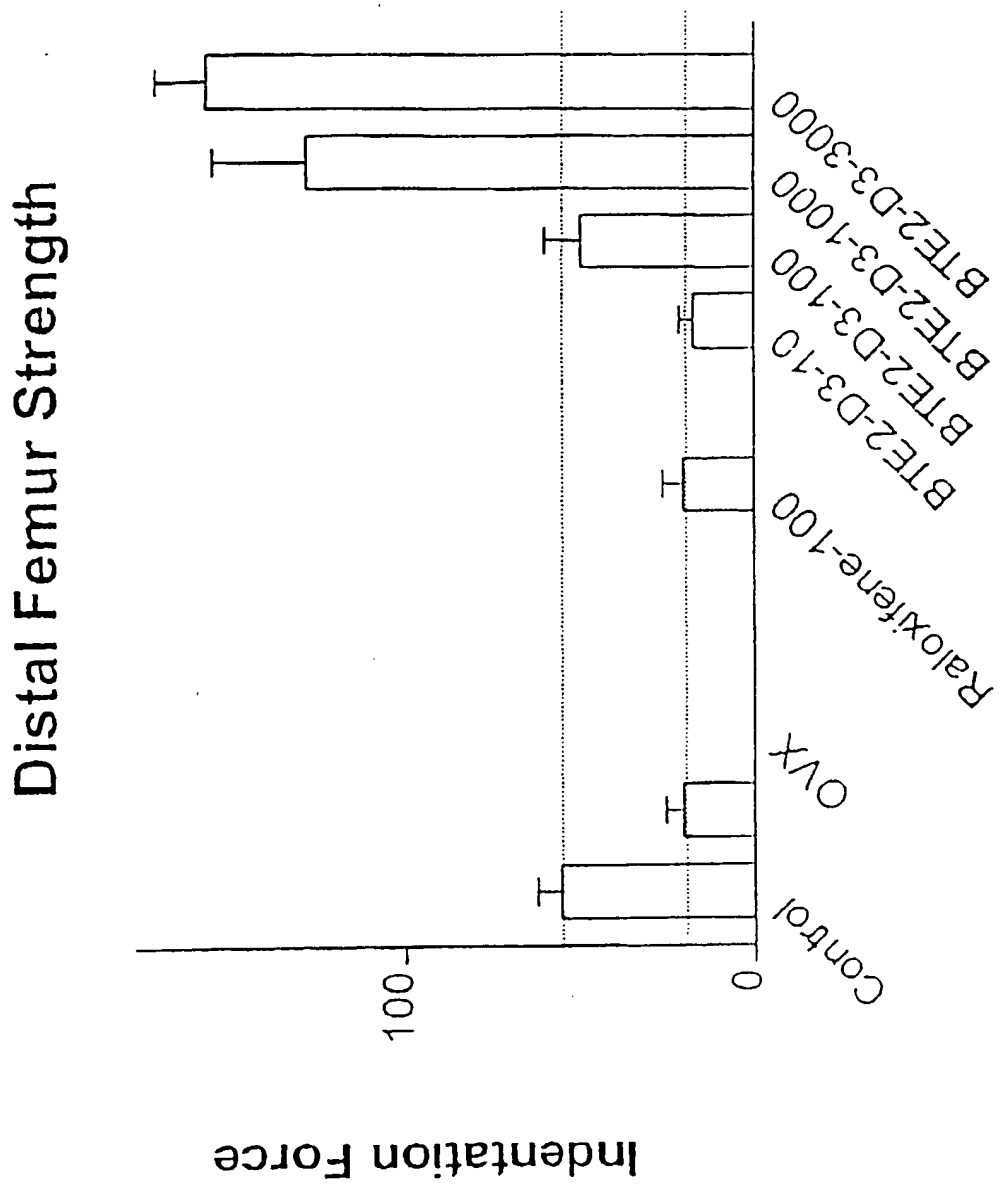
Distal Femur Strength
Indentation Force
100
0
Control
OVX
Raloxifene-100
BTE2-D3-10
BTE2-D3-100
BTE2-D3-1000
BTE2-D3-3000

FIGURE 2

# INTERNATIONAL SEARCH REPORT

Inter ›nal Application No
PCT/US 00/11655

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07J41/00 C07J51/00 A61K31/565 C07C237/44 A61P3/14
A61P19/10

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07J A61K C07D C07C A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data, PAJ, BEILSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 28 September 2000 | 23/10/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016 | Watchorn, P |

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Form PCT/ISA/210 (second sheet) (July 1992)

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| Y | page 648, paragraph 1<br>page 649, compounds III and IX<br>---<br>-/-- | 1-65 |

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# INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 00/11655

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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| X | CHEMICAL ABSTRACTS, vol. 53, no. 0, 1959 Columbus, Ohio, US; abstract no. 8054d, R. BRANCHINI ET AL: "Some derivatives of p-orsellinic acid" column 2; XP002146783 abstract & ANN. CHIM., vol. 48, 1958, pages 819-825, ROME --- | 46, 48-50,56 |
| X | E. G. BRAIN ET AL: "Derivatives of 6-Aminopenicillanic Acid. Part IV. Analogues of 2,6-Dimethoxyphenylpenicillin in the Naphthalene and Quinoline Series" JOURNAL OF THE CHEMICAL SOCIETY, 1963, pages 491-497, XP002146779 LETCHWORTH GB page 493, last paragraph -page 494, paragraph 1 --- | 46,47,56 |
| X | BARTON, D. H. R. ET AL: "Synthesis of tetracycline. VI. Oxidation and reduction of potential ring A precursors" J. CHEM. SOC. C (1971), (12), 2204-15 , XP002146780 page 2204, column 1, paragraph 3 ----- | 46,48, 49,56 |

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Interr nal Application No
PCT/US 00/11655

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